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Pyloric Stenosis of Infants

A Statistical Analysis of Sixty Cases

By Wilburt C. Davison, M.D.[1]
Baltimore, Maryland

ALTHOUGH much of the following analysis may seem to be "a painstaking delineation of the obvious," yet it illustrates the distribution of cases and deaths in relation to several factors. Definite conclusions cannot of course be drawn from this small series, but nevertheless many suggestive deductions are possible. All of the 60 patients who form the basis of this analysis had unmistakable signs of pyloric stenosis. Thirty-two of them were treated medically and will be referred to as the medical group. The medical treatment of pyloric stenosis in infancy will be discussed in the second paper of this series (1). The surgical group consists of 28 patients who were operated upon (the Fredet-Rammstedt operation in 23 and a posterior gastroenterostomy in 5). The surgical treatment of pyloric stenosis will be described in the third paper of this series (2).

These sixty infants represent all of the cases of this condition which were admitted to the Harriet Lane Home from October, 1913, to June, 1922. Patients in whom the diagnosis was doubtful—the group usually described as suffering from "pylorospasm"—were not included. Since this paper was written, 6 other patients suffering from pyloric stenosis have been admitted. Two of these were treated medically and 4 were operated upon. One of the latter died.

As shown in this analysis, the case distribution[2] of the combined medical and surgical groups indicates the relation of various factors to pyloric stenosis, while that of the individual medical and surgical groups demonstrates the similarity or the dissimilarity of the cases in each group.

Symptoms and physical signs (Table I). The four cardinal symptoms and physical signs of pyloric stenosis are visible gastric peristalsis, projectile vomiting, a palpable tumor of the pylorus, and constipation. Although the presence of the first two of these is usually sufficient for a diagnosis of pyloric stenosis, the existence of all four renders the condition unmistakable. Since two-thirds of the infants operated upon had all four of the symptoms or signs, while they were all present in only a little more than one-third of the medical group, it might

[1] From the Department of Pediatrics, Johns Hopkins University, and the Harriet Lane Home, Johns Hopkins Hospital, Baltimore, Maryland.

[2] Case distribution refers to the percentage of the total number of patients suffering from pyloric stenosis that occurs in each group.

be assumed that a greater number of the patients in the surgical group had a more extreme degree of pyloric stenosis. The fact that approximately one-third of the medical group had only two of these symptoms or signs in those patients who had only the first three of these symptoms, or signs, and who instead of being constipated had diarrhoea or normal stools. The occurrence of more cases of this category in the medical group than

TABLE I

The distribution of cases and deaths in relation to symptoms and physical signs

SYMPTOMS AND PHYSICAL SIGNS	MEDICAL AND SURGICAL GROUPS COMBINED				MEDICAL GROUP ALONE				SURGICAL GROUP ALONE			
	Number of patients	Case distribution*	Deaths	Case fatality†	Number of patients	Case distribution*	Deaths	Case fatality†	Number of patients	Case distribution*	Deaths	Case fatality†
		per cent		per cent		per cent		per cent		per cent		per cent
Visible gastric peristalsis only	1	1.7	0	0	1	3.1	0	0	0	0	0	0
Visible gastric peristalsis and projectile vomiting only	12	20.3	0	0	11	34.4	0	0	1	3.7	0	0
Visible gastric peristalsis, projectile vomiting and constipated stools only	8	13.6	1	8.3	3	9.3	0	0	5	18.5	1	20
Visible gastric peristalsis, projectile vomiting, and a palpable tumor of the pylorus only	8	13.6	3	37.5	5	15.6	1	20	3	11.1	2	66.7
Visible gastric peristalsis, projectile vomiting, constipated stools and a palpable tumor of the pylorus	30	50.8	3	10	12	37.5	2	16.7	18	66.8	1	5.5
Undetermined‡ (History incomplete)	1	+	0	0	0	0	0	0	1	+	0	0
Total	60	100	7	11.7	32	100	3	9.3	28	100	4	14.3

* Case distribution is calculated with reference to the number of patients in each of the three groups, i.e., medical and surgical groups combined, medical group alone and surgical group alone.

† Case fatality is calculated with reference to the number of patients in each separate classification, i.e., male white, female white, etc.

‡ These patients are not included in the calculation of case distribution.

might also appear to strengthen this assumption. However, the case fatality rates[3] in each group were highest in the surgical group, I believe, counterbalanced the smaller number of infants with all four features, so that the medical and surgical groups probably contained cases of pyloric stenosis of approximately equal severity.

Color and sex (Table II). Pyloric stenosis was slightly more than twice

[3] The case fatality rates are in all cases specific for the items of the classification in which they appear, *e.g.*, in Table II, 6 of the 36 male white infants died—a case fatality rate of 16.7 per cent.

as frequent in males as in females among the white infants in this series of cases, but in the colored the condition was practically evenly distributed among males and females. In the series of 163 patients suffering from pyloric stenosis who were treated for reasons which will presently appear. It merely describes one attribute of the present series.

The case *incidence*[4] of pyloric stenosis in male infants in the whole experience of the Harriet Lane Home has been nearly twice as great as that

TABLE II

The distribution of cases and deaths in this series, and the general case incidence rate of pyloric stenosis in the experience of the Harriet Lane Home in relation to sex and color

SEX AND COLOR	MEDICAL AND SURGICAL GROUPS COMBINED					MEDICAL GROUP ALONE				SURGICAL GROUP ALONE			
	Number of patients	Case distribution	Case incidence per 1000 patients*	Deaths	Case fatality	Number of patients	Case distribution	Deaths	Case fatality	Number of patients	Case distribution	Deaths	Case fatality
		per cent			per cent		per cent		per cent		per cent		per cent
Male, white	36	60.0	2.82	6	16.7	17	53.1	2	11.8	19	67.8	4	21.0
Female, white	17	28.3	1.48	1	5.9	11	34.3	1	9.1	6	21.4	0	0
Male, colored	3	5.0	0.65	0	0	2	6.25	0	0	1	3.6	0	0
Female, colored	4	6.6	0.97	0	0	2	6.25	0	0	2	7.1	0	0
White (both male and female)	53	88.3	2.18	7	13.2	28	87.5	3	10.7	25	89.3	4	16.0
Colored (both male and female)	7	11.6	0.81	0	0	4	12.5	0	0	3	10.7	0	0
Male (both white and colored	39	65.0	2.25	6	15.4	19	59.3	2	10.5	20	71.4	4	20.0
Female (both white and colored	21	35.0	1.35	1	4.8	13	40.6	1	7.7	8	28.6	0	0
Total	60	100.0	1.83	7	11.7	32	100.0	3	9.3	28	100.0	4	14.3

* Case incidence is calculated from the numbers of male white, female white, male colored and female colored patients admitted for all conditions to the Harriet Lane Home Dispensary for the first time (32,892 new patients) 1912–1922 inclusive.

surgically (Fredet-Rammstedt operation), reported by Goldbloom and Spence (3), there were over four times as many males as females.

Among the infants of the present series, there were nearly eight times as many whites as colored, regardless of sex. This proportion is not to be taken as indicating the greater susceptibility of whites to this condition,

[4] Case incidence refers to the number of cases of pyloric stenosis which have occurred in every 1000 patients of the same sex, color, etc., who have been admitted for various conditions to the Harriet Lane Home. In so far as the admissions to the Harriet Lane Home may be regarded as a fair sample of an urban general population, the case incidence figures in this paper may be taken as representative pyloric stenosis morbidity rates for infants, specific for sex, age and color.

among females in the case of white infants, but among the colored the case incidence has been nearly the same in both sexes. The sex relation of the total morbidity rates (case incidence) is approximately the same as that shown in the case distribution of this series, since the numbers of male and female patients admitted for various conditions to the Harriet Lane Home for the past ten years were almost the same. In this decade, pyloric stenosis per thousand admissions has been two and a half times as high among white infants as among colored, regardless of sex. This demonstrates what must be an approximately correct measure of the greater liability of white infants to pyloric stenosis as compared with colored, under the environmental conditions and habits of life peculiar to the two races in Baltimore. The ratio of the racial morbidity incidence rates is different from that of the racial case distribution rates because the number of white infants admitted for various conditions to this hospital for the past ten years has been nearly three times that of colored patients.

No colored infants in this series died. Among the white patients, the case fatality rate was approximately three times greater in males than in females. In the medical group it was slightly higher in males than in females; while in the surgical group all of the fatalities were in males. These figures are similar to those of Goldbloom and Spence (3) who found the case fatality rate to be twice as high in males as in females.

Age at onset.[5] In nearly three-fourths of the patients, the initial symptoms of pyloric stenosis began during the first three weeks of life. Thirty-one per cent of the infants had vomited since birth, and 41 per cent had developed symptoms between the ages of 2 and 3 weeks.

The median ages at onset in each of the groups were similar, that of the medical group being 16.5 days and of the surgical 15 days. The minimum age at onset in both the medical and surgical groups was birth; the maximum age in the former was 56 days and in the latter 240 days. The infant whose age at onset was 8 months (240 days) had been normal until that time and then had gradually developed all four of the cardinal signs and symptoms of pyloric stenosis.

In the medical group, none of the patients who had vomited since birth died, while in the surgical group the case fatality rate was 25 per cent in infants whose symptoms had dated from birth. When all of the cases were analyzed together (medical and surgical groups combined) the case fatality rate was not affected by the age at onset for it was the same (12 per cent) whether the symptoms had begun at birth, during the second week of life or after the fifth week.

Birth order. Nearly half of the total number of patients in each group were first children. The case fatality rate of both the medical and surgical groups was much higher among first children.

Type of onset. Contrary to the usual description of pyloric stenosis, the symptoms in more than half of the

[5] To facilitate publication, 22 of the 27 tables on which this study is based have been omitted. I am indebted to Dr. Raymond Pearl for his assistance in the compilation of these data.

patients began gradually. These infants vomited once or twice a day for the first few days. The frequency then increased until everything fed was vomited. The vomiting also increased in force until it became definitely projectile. A sudden onset is an important factor in the case fatality rate of pyloric stenosis, for it was markedly higher among patients whose symptoms began suddenly, being 25 per cent in those of sudden onset and only 3 per cent among patients whose onset was gradual. None of the 20 patients whose onset was gradual and who were treated medically died and only 1 of the 15 (7 per cent) who were operated upon succumbed.

TABLE III

The distribution of cases and deaths in relation to the general condition of the patient on admission to the Harriet Lane Home

General condition of patient on admission to the Harriet Lane Home	Medical and surgical groups combined				Medical group alone				Surgical group alone			
	Number of patients	Case distribution	Deaths	Case fatality	Number of patients	Case distribution	Deaths	Case fatality	Number of patients	Case distribution	Deaths	Case fatality
		per cent		per cent		per cent		per cent		per cent		per cent
Good	48	80.0	4	8.3	26	81.2	0	0	22	78.5	4	18.2
Bad	12	20.0	3	25.0	6	18.8	3	50.0	6	21.5	0	0
Total	60	100.0	7	11.7	32	100.0	3	9.3	28	100.0	4	14.3

Age at onset and type of onset. In practically all of the infants whose symptoms began during the first week of life, the type of onset was gradual. Among those in whom the condition began during the second to the third week, gradual and sudden types of onset were equally distributed. The onset was sudden in two-thirds of the infants who were normal until after the fourth week. In other words, the older the child, the more frequently was the onset sudden. In the medical group, the case fatality rate was highest among infants whose symptoms commenced suddenly at the age of 2 to 3 weeks, while in the surgical group, except for the fact that the only patient whose symptoms began suddenly at the age of 1 week died, the highest case fatality rate was in infants whose symptoms commenced suddenly at the age of 4 to 6 weeks.

Age at onset, birth order and type of onset. The most frequent occurrence of cases in the medical group was in infants who were not first-born and whose symptoms began gradually at birth. In the surgical group, the most frequent occurrence of cases was among patients, who likewise were not first-born but whose symptoms commenced suddenly at the age of 2 to 3 weeks.

General condition of the patient (Table III). In this series, patients who were in good condition on ad-

mission were four times as frequent as those in bad condition. None of the patients who were in bad condition when operated upon died, while 3 of the 6 whose condition was bad and who were treated medically succumbed. These 3 patients were moribund on admission and were refused operation by the surgeons because of their hopeless condition. For this reason only they are included in the medical group. None of the patients in the medical group who were in good condition died, whereas a fifth of the infants who were in good condition when operated upon succumbed. Two deductions seem possible from these data; first, that for patients in bad condition, an immediate operation is advisable, even if the case is apparently hopeless. Surgery offers the better chance for these patients. Second, if the infant is in good condition, an operation should be deferred until medical treatment has been given a trial, for any operative procedure in children has an inherent mortality. Some of the factors to be taken into consideration before deciding upon the type of treatment of the individual patient will be discussed in the next paper (1).

Interval between onset and admission to hospital. Thirty-eight per cent of the infants in this series were admitted to the Harriet Lane Home within three weeks of the onset of their symptoms. The only difference in this respect between the types of cases in the medical and surgical groups was that in the former there was an equal frequency of patients (one fourth of all in the group) who were admitted to the hospital within three weeks of the onset of their symptoms and of those admitted 9 to 12 weeks after onset, while in the surgical group somewhat more than half of the infants were admitted to the hospital within three weeks after onset.

The length of time between onset and admission apparently was an unimportant factor in the case fatality rate. This is contrary to the data published by Goldbloom and Spence (3). The case fatality rate in their series of surgical cases was 13 per cent in patients admitted within four weeks of onset, and 35 per cent in those admitted after a longer period. When analyzed in a similar way, the case fatality rate in the combined medical and surgical groups of the present series was 13.8 per cent in infants admitted within four weeks after onset and 9.7 per cent in those admitted after a longer period. These percentages were practically the same in the medical and surgical groups.

Interval (between onset and admission) and the general condition of the patient. The proportion of the patients in good condition to those in bad condition was not markedly affected by the length of the interval between onset and admission. This observation is contrary to the usual supposition that the longer the interval between onset and admission, the greater will be the proportion of patients in bad condition. This lack of relation may possibly be explained on the basis that the majority of the patients in bad condition do not survive an interval between onset and admission longer than three weeks.

Diet. The frequency, in this series, of patients who were breast fed exclusively, or who had been given rational supplementary feedings, was twice

that of those who were badly fed, *i.e.*, 66.6 per cent and 33.3 per cent respectively. These figures are practically identical with those published by Goldbloom and Spence (3), namely, 65 per cent and 35 per cent. It is more probable that this ratio indicates the proportion of infants who are breast fed at this age, rather than a greater susceptibility of breast fed infants to pyloric stenosis.

In the medical group the fatalities occurred only in patients who were badly fed, while in the surgical group they were confined to infants who had received a proper diet. The reason for this difference is probably the same as that advanced above to explain the relation of the general condition of the patient to the case fatality rate. The relation of the patient's diet to the case fatality rate in the present surgical group is exactly opposite to that of the surgical series analyzed by Goldbloom and Spence, *i.e.*, among their breast fed infants, the case fatality was 11.3 per cent and in those artificially fed, it was 35.0 per cent.

General condition and diet. In the combined medical and surgical groups and in the surgical group alone the highest case frequency occurred among infants who were in good condition and who were breast fed, while, in the medical group alone, the highest case frequency was among infants who were in good condition but who had been badly fed. All of the fatalities in the medical group were in badly fed infants who were in bad condition, while all of the deaths in the surgical group group occurred in infants who were rationally fed and who were in good condition. This difference in case fatality emphasizes again that medical treatment is almost useless for patients in bad condition, whereas surgery, although it may save infants in bad condition, may yet be responsible for the deaths of infants who are in good condition.

Birth weight. In the surgical as well as in the medical group of this series, infants of normal birth weight were the most frequent. The fatalities in the medical group occurred only in infants who weighed from 7 to 9 pounds at birth, while in the surgical group patients whose birth weight was from 5 to 7 and 7 to 9 pounds had approximately the same case fatality rates. No fatalities occurred in infants who were overweight at birth.

Loss or gain in weight between birth and admission to hospital. Infants who had lost from ½ to 3 pounds of their birth weight formed 58 per cent of the medical group and 95 per cent of the surgical group. As a decision to operate was usually based upon a history of persistent loss of weight, it is natural that the surgical group should be largely confined to infants who had failed to gain in weight. The case fatality rate in both groups was highest among patients who had lost the most weight. No fatalities occurred among infants who had gained in weight since birth.

Percentage of birth weight gained or lost before admission to hospital. Seventy-four per cent of the patients in the combined medical and surgical group had lost from 1 to 35 per cent of their birth weight and the remainder had gained from 1 to 70 per cent in weight. The deduction from this analysis is practically identical with that expressed above, namely, that the case fatality rate was highest

among infants who had lost the greatest percentage of their birth weight.

Birth weight and gain or loss in weight since birth. When the data were analyzed to study the distribution of cases in relation to both of these factors, it was found that infants of normal birth weight predominated in both groups, but those who had lost weight between birth and admission were more frequent in the surgical than in the medical group. The case fatality rate was highest among patients who had lost weight, especially among those of normal birth weight. Loss of weight between birth and admission was apparently a more important factor in case fatality than low birth weight.

Weight and age. Infants who weighed from 5 to 7 pounds on admission to the Harriet Lane Home were of the most frequent occurrence in both the medical and surgical groups. The fatalities, with one exception, were limited to these underweight infants. In the medical group, the greatest frequency was of infants who were over 6 weeks of age on admission to the hospital, while the reverse was true in the surgical group. Age on admission had no apparent relation to the case fatality rate.

Month of onset (seasonal variation). The case incidence relative to the whole Harriet Lane Home experience and the case frequency of the combined medical and surgical groups of the present series were lowest from March to September. There was no apparent seasonal variation of the case fatality rate.

Age at time of operation. The cases and also the deaths in the surgical group were about equally divided between infants who were less than six weeks old at the time of operation and those who were over this age.

Duration of hospital stay of patients who recovered. The cases in the medical group were about equally divided between patients who remained in the hospital one, two and three months, while in the surgical group, nearly three-fourths of the patients stayed in the hospital less than one month. These data (Table IV) illustrate one of the main objections to the medical treatment of pyloric stenosis, namely, the long hospitalization required with its attendant exposure to infections of various kinds. The median duration of the hospital stay of patients treated medically was 53 days as contrasted with 19 days for those of the surgical group.

Case fatality rate among cured patients after discharge from the hospital. Of the 23 patients who were discharged as cured after medical treatment and whose later course was known, 5 died, while of the 17 who were cured surgically only 2 were known to have succumbed. All of these 7 patients died within less than one year after discharge from the hospital as a result of accident, pneumonia or other causes which bore no relation to pyloric stenosis.

SUMMARY (TABLE V)

An analysis has been made of the distribution of cases and deaths in a series of 60 cases of pyloric stenosis (32 patients treated medically and 28 surgically) in relation to the following factors: symptoms, physical signs, sex, color, age at onset, birth order, type of onset, general condition of the patient, interval between onset and admission

TABLE IV

Maximum, minimum and median duration of hospital stay of patients who recovered from pyloric stenosis

DURATION OF HOSPITAL STAY	MEDICAL AND SURGICAL GROUPS COMBINED	MEDICAL GROUP ALONE	SURGICAL GROUP ALONE		
			Total hospital stay	Hospital stay before operation	Hospital stay after operation
	days	*days*	*days*	*days*	*days*
Maximum duration	96	234	96	23	75
Minimum duration	0	5	8	0	8
Median duration	35	53	19	3	13

TABLE V

Summary. The distribution of cases and deaths in this series in relation to various factors

FACTOR	CASE DISTRIBUTION (MEDICAL AND SURGICAL GROUPS COMBINED)	CASE FATALITY (MEDICAL AND SURGICAL GROUPS COMBINED)
Symptoms and physical signs	Highest among patients who presented all four cardinal features of pyloric stenosis	Highest among patients who were not constipated and especially high among those who had diarrhoea
Sex and color	Highest in white males	Highest in white males
Age at onset	Highest in infants 2 to 3 weeks of age at onset	No apparent relation
Birth order	Nearly half were first-born	Highest among first-born
Type of onset	Highest among patients whose onset was gradual. The frequency of a sudden onset increased with the age at onset	Highest in patients whose onset was sudden
General condition of the patient	Highest among patients in good condition	Highest among patients in bad condition in the medical group and among those in good condition in the surgical group
Interval between onset and admission to the hospital	Highest among patients admitted within 3 weeks of onset	No apparent relation
Previous diet	Highest among infants who were not exclusively breast fed	No apparent relation
Birth weight	Highest among infants of normal birth weight	Lowest in infants who were above normal weight at birth
Loss or gain in weight since birth	Highest among infants whose admission weight was less than their birth weight	Highest among infants whose loss of weight was greatest
Weight and age on admission	Highest in young infants of low weight on admission	Highest in infants weighing less than 7 pounds on admission.
Month of onset (seasonal variation)	Lowest during summer months	No apparent relation
Age at time of operation	No apparent relation (surgical group)	No apparent relation (surgical group)

to the hospital, previous diet, birth weight, loss or gain in weight since birth, weight and age on admission to the hospital, and the month of onset. The distribution of cases and deaths in relation to the age at operation of the 28 surgical patients has also been analyzed. In addition, among the patients who recovered from pyloric stenosis the following factors were studied: duration of hospital stay, and course after discharge from the hospital.

CONCLUSIONS

The frequency of pyloric stenosis, as far as can be determined from a study of sixty patients, of whom 32 were treated medically and 28 were operated upon, was highest among white, male, first-born infants of normal birth weight. The case fatality rate was highest among those who were not constipated, whose onset was sudden, who weighed less than seven pounds at birth and on admission, and who had lost the most weight since birth. No fatalities ocurred among infants who were overweight at birth or who had gained in weight between birth and admission to the hospital.

The fact that the case fatality rate was slightly higher in the surgical than in the medical group is, of course, no reason that all infants with pyloric stenosis should be treated medically, but it is a very good argument against operating upon all of these patients as soon as the diagnosis is made, as some surgeons have maintained. It should be mentioned that the 3 patients of the medical group who died were moribund on admission and were refused operation by the surgeons because of their hopeless condition. If they had not been included in the medical group, the medical case fatality rate would have been zero.

The more obvious deductions to be drawn are:

(1) To operate upon all patients who are in such bad condition on admission to the hospital that delay would be fatal,

(2) To make a trial of medical therapy in infants who are not in a critical condition and

(3) To operate upon patients in whom medical therapy is not producing any improvement.

By selective therapy, rather than by exclusively surgical or medical treatment, the total case fatality rate can be reduced.

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Studies on the Biology of the Streptococcus erysipelatis

II. Demonstration of Antigenic Relationships Among Strains of Streptococcus erysipelatis by Intradermal Protection Tests[1]

By Konrad E. Birkhaug

From the Biological Division of the Medical Clinic, The Johns Hopkins Hospital

By means of agglutination and agglutinin absorption (1, 2) it was shown that 91.2 per cent of the cultures isolated from typical erysipelatous lesions fell immunologically into one group, and that this group of hemolytic streptococci could be clearly differentiated from the group of streptococci associated with scarlet fever and numerous other streptococcic infections.

There has been much debate concerning the reliability of such immune reactions as agglutination and agglutinin absorption when used for the purpose of determining type specificity among the large family commonly called Streptococcus hemolyticus. The enormous literature on the subject of bacterial agglutination is replete with inconsistencies and contradictions. The main criticism raised against the validity of the results obtained by agglutination and agglutinin absorption has been the spontaneous agglutinability of streptococci in suspensions of physiological saline solution and beef infusion broth twenty-four hours after incubation. Mellon (3) has concluded that this phenomenon of flocculation of bacteria can be averted by the action of equilibrated solutions of electrolytes in proportions similar to those of physiologically "balanced" or equilibrated solutions. He also found that the prompt spontaneous agglutinability in sodium chloride of the diphtheroid organism studied could be completely reversed when grown at room temperature (approximately 20°C.). With the media employed, this reversibility was associated with morphologic and staining changes. The evidence, according to Mellon, was presumptive that the adaptation of the culture to 20°C. is accomplished through the mechanism of pleomorphism, whereby the culture can be segregated into two physiologically distinct, albeit reversible, phases of growth. These observations have been corroborated in our work on the agglutination and agglutinin absorption of Streptococcus scarlatinae and Streptococcus erysipelatis. This entire subject of agglutination and agglutinin absorption as criteria of

[1] Read before the American Association of Immunologists, May 4, 1925. Received for publication, May 7, 1925.

antigenic differentiation among groups of bacteria has recently been thoroughly reviewed and severely criticized by Krumwiede, Cooper and Provost (4), Breinl and Fischer (5), Taylor (6), and numerous other investigators.

In view of adverse criticism concerning the validity of agglutination and agglutinin absorption of bacteria as reliable methods for determining type specificity or immunological relationships among groups of bacteria, it became necessary to resort to the method suggested by the work of Rivers and Tillett (11) for the demonstration of antigenic relationships of bacteria *in vivo*.

That the skin possesses a special biological function not shared by other tissues in the body has been suggested by Besredka (7), Gay (8), Mironesco (9) and the author's observations concerning the blanching phenomenon in scarlet fever (10). Rivers and Tillett (11) have shown that small areas of skin infiltrated with an immune monovalent streptococcic serum produced local protection against the culture of hemolytic streptococcus employed for the production of the serum. These authors have also concluded that areas of skin infiltrated with either normal serum or beef infusion broth proved more refractory than normal skin to subsequent infection with hemolytic streptococci inoculated 24 hours later. In our studies it was demonstrated that intradermal infiltration of normal serum or beef infusion broth did not offer enough protection against the injurious activity of intradermally injected hemolytic streptococci to be of any practical significance as non-specific factors in the following experiments. On the other hand, it was demonstrated in our studies on local immunity in relation to the production of experimental erysipelas (12) that adequate protection was obtained with 0.005 cc. of an immune erysipelas serum against 0.1 cc. of the homologous culture of Streptococcus erysipelatis, and with 0.01 cc. immune serum against 0.1 cc. of heterologous cultures of erysipelatous streptococci. It has also been shown that no adequate protection was produced with immune erysipelas serum against infections of unrelated, non-erysipelas cultures of hemolytic streptococci isolated from cases with cellulitis, dermatitis, empyema, peritonitis, sinusitis, scarlet fever and tonsillitis. The chief value of these findings depended on the fact that the *in vivo* results of the protection-tests confirmed in the main the *in vitro* results of the agglutination and agglutinin absorption reactions. The results by both these two methods suggest that the groups of hemolytic streptococci isolated from erysipelatous lesions fall antigenically into one distinct group, which can be differentiated by these same methods from the group of hemolytic streptococci isolated from the throat secretions of scarlet fever patients, as well as from other streptococcic infections. In view of these facts, it seemed that the skin of normal rabbits, rendered passively immune with known streptococcic anti-sera, might be employed as a medium in which to demonstrate antigenic relationships among strains of Streptococcus hemolyticus.

METHOD AND MATERIAL

The rabbit's chest and abdomen were depilated on both sides of the

body until the shaved area measured about 8 x 12 cm. The shaved skin areas were then divided into small squares measuring about 4 square centimeters. Each square was infiltrated with 0.1 cc. of an immune serum. The injection of the serum was made intradermally with a Luer tuberculin-syringe and a 27-gauge needle. Twenty-four hours were allowed to elapse for the fixation of the immune bodies in the infiltrated cutaneous areas. Into the center of each passively immunized area was injected intradermally 0.1 cc. of a hemolytic streptococcal emulsion which was made from a 24-hour blood-agar slant-culture brought into suspension with 10 cc. of Locke's solution. For the investigation of local passive immunity against the infection of hemolytic streptococci a series of 5 immune monovalent erysipelas sera and one monovalent scarlet fever serum were employed. Normal rabbit serum, free from natural agglutinins for the cultures of streptococci used in these experiments, was chosen for control. A series of 22 cultures of hemolytic streptococci isolated from a variety of streptococcic infections were injected separately into the skin areas passively immunized 24 hours previously. For the sake of accuracy in the interpretation of the results, each strain of Streptococcus hemolyticus was tested in double series on both sides of the body. The preparation of the immune sera and the source of the cultures employed in these experiments have been fully described in a previous report (2). The nomenclature of the sera and the strains are identical with that employed in the same report.

LOCAL PASSIVE IMMUNITY AS AN AID IN DEMONSTRATING ANTIGENIC RELATIONSHIPS AMONG STRAINS OF STREPTOCOCCUS HEMOLYTICUS

The following experiments were performed in order to demonstrate by intradermal passive immunity whether antigenic relationships exist among strains of Streptococcus hemolyticus.

A series of 25 normal rabbits whose skin was susceptible to infection with hemolytic streptococci, when these were injected intradermally, and whose blood-serum was free from natural agglutinins for the cultures of streptococci employed, were selected for the following experiments. The depilated skin on each of the animals was divided into seven squares of about 3 to 4 square centimeters. Into the center of each area 0.1 cc. of one of the seven sera employed was infiltrated intradermally. Care was taken to infiltrate the skin cutaneously, forming a bleb about 1 to 1.5 cm. in diameter in the upper layers of the skin. Early failures in producing local passive immunity were apparently due in a large measure to subcutaneous infiltration of the immune serum rather than to intracutaneous injection. The seven areas immunized locally represented each one of the following sera: anti-erysipelas sera: "B_3," "C_1," "C_4," "C_5," "C_7;" anti-scarlatinal serum: "SF_{22};" and normal rabbit serum. Twenty-four hours later 0.1 cc. of one strain of hemolytic streptococcal suspension was injected intradermally into the center of each of the seven immunized areas on either side of the body. Twenty-two strains of streptococci isolated from a variety of infec-

tions were tested in this way in a series of 25 normal rabbits. Only one strain of Streptococcus hemolyticus was employed in any one rabbit. During the following seven days the animals were watched carefully to see if infection occurred from the injected streptococci. It was observed that the erysipelatous streptococcal suspensions did not produce lesions in the areas infiltrated with the anti-erysipelas sera "B_3," "C_1," "C_4," "C_5" and "C_7" except in the areas inoculated with strain "C_6," where typical erysipelatous lesions developed which extended beyond the borders of each individual area. Sporadic lesions developed in four areas inoculated with erysipelatous streptococci, but they occurred in only one out of five passively immunized areas.

It was observed that 13 out of 14 cultures of erysipelatous streptococci, or 93 per cent, produced no visible lesions in the skin locally immunized with anti-erysipelas sera. Strain "C_6" alone produced lesions in areas infiltrated with any of the five immune erysipelas sera. This observation is in accord with the results obtained with the same culture *in vitro* when it failed to be agglutinated by any one of the seven immune erysipelas sera exposed to it, or to absorb the agglutinins from any one of these immune sera (2).

Scarlet fever immune serum did not inhibit the pathogenic activity of any one of the 14 cultures of erysipelatous hemolytic streptococci. Normal rabbit serum failed to offer any appreciable "non-specific protection."

On further investigation of the antigenic nature of eight cultures of non-erysipelas hemolytic streptococci isolated from cases of cellulitis, dermatitis, empyema, acute nasal sinusitis and scarlet fever, it was found that no protection against infection was offered these streptococci by any one of the five monovalent anti-erysipelas sera, except in the instance of strain "N_2." This strain was isolated from a case of acute nasal sinusitis in which a history was obtained of recurrent facial erysipelas. Complete local protection was demonstrated against injurious action of this culture by four anti-erysipelas monovalent sera. The result *in vivo* was in complete agreement with the reaction obtained by agglutination and agglutinin absorption (2) of this culture.

The anti-scarlatinal monovalent serum inhibited the injurious activity in the skin of three cultures of scarlatinal hemolytic streptococci.

DISCUSSION

Observations made in the course of the experiments reported in this paper demonstrate that the phenomenon of local "tissue resistance" or local passive immunity offers distinct and practical advantages as a means of measuring specific antibody content of an immune serum when this is employed as local protection against the injurious activity of the homologous culture of the hemolytic streptococcus that produced the immune serum, as well as for detection of specific antigenic relationships of heterologous cultures of the same immunological group of streptococci. By employing the method of intradermal infiltration of an immune serum 24 hours previous to the inoculation of streptococci into the area passively immunized, the erysipelas strains have been separated from

other hemolytic streptococci and shown to fall into one immunological group, which may thus be readily distinguished from the specific group of streptococci associated with scarlet fever and other streptococcic infections.

use of *in vivo* protection tests. Such procedure of investigation of existing antigenic relationships or variants of bacteria may conceivably serve to correlate the results obtained by the two methods and may prevent false interpretation of the variable immunologi-

TABLE 1

Intradermal protective power of immune erysipelas sera, scarlet fever serum, and normal rabbit serum when injected into the skin 24 hours previous to inoculations of hemolytic streptococci

STRAINS	TYPE OF STRAIN	DOSE OF CULTURE	IMMUNE ERYSIPELAS SERA					SCARLET FEVER SERUM	NORMAL RABBIT SERUM
			"B_3"	"C_1"	"C_4"	"C_5"	"C_7"		
			Seven areas were inoculated with 0.1 cc. each.						
		cc.							
B_3	Erysipelas	0.1	0	0	0	0	0	+++	++++
C_1	Erysipelas	0.1	0	0	0	±	0	+++	+++
C_2	Erysipelas	0.1	0	0	0	0	0	+++	++++
C_3	Erysipelas	0.1	0	+	0	0	0	+++	++++
C_4	Erysipelas	0.1	0	0	0	0	0	++++	++++
C_5	Erysipelas	0.1	0	0	0	0	0	+++	+++
C_6	Erysipelas	0.1	+++	+++	++	++	+++	+++	++
C_7	Erysipelas	0.1	0	0	0	0	0	++	++
C_8	Erysipelas	0.1	0	++	0	0	0	+++	+++
C_9	Erysipelas	0.1	0	0	0	0	0	++	++
C_{10}	Erysipelas	0.1	0	0	0	0	0	+++	++++
C_{32}	Erysipelas	0.1	0	0	0	0	0	++++	++++
C_{33}	Erysipelas	0.1	0	0	++	0	0	++++	++++
C_{34}	Erysipelas	0.1	0	0	++	0	0	++++	++++
D_2	Cellulitis	0.1	+++	+++	++++	+++	+++	+++	++++
D_4	Dermatitis	0.1	++	++	+++	++	++	++	++
E_4	Empyema	0.1	+++	+++	+++	++++	++++	++++	+++
N_2	Sinusitis	0.1	0	0	0	++	0	++++	++++
N_4	Sinusitis	0.1	+++	+++	++++	++++	+++	+++	++++
SF_{32}	Scarlet fever	0.1	++	++	+++	+++	++	0	+++
SF_{40}	Scarlet fever	0.1	+++	++	+++	++	++	0	+++
SF_{58}	Scarlet fever	0.1	++	++	+++	++	++	0	+++

0 signifies complete protection;++++ indicates cutaneous lesion measuring 4 cm. in diameter or over, +++ 3 cm., ++ and + 2 cm. and 1 cm., respectively, and ± doubtful lesion.

The criticism and difficulties encountered by such *in vitro* methods as agglutination and agglutinin absorption, do not, therefore apply, to the experiments made *in vivo*. Hence, one might readily verify the results obtained by *in vitro* methods by the

cal reactions of agglutination and agglutinin absorption.

SUMMARY

1. Intradermal infiltration of the skin of rabbits with immune erysipelas serum 24 hours previous to the injec-

tion of hemolytic streptococci of erysipelas and non-erysipelas origin, offered adequate protection against 93 per cent of cultures of erysipelatous streptococci. Immune erysipelas serum offered no local passive immunity against the injurious action of cultures of non-erysipelatous hemolytic streptococci.

2. Intradermal infiltration with immune scarlatinal serum 24 hours previous to the injection of hemolytic streptococci isolated from throat secretions of scarlet fever patients offered local protection against the injurious action of the homologous and heterologous cultures of scarlatinal streptococci. Immune scarlatinal serum offered no local passive immunity against the injurious action of cultures of non-scarlatinal hemolytic streptococci.

The results of the experiments are shown in Table I, and in summary constitute either protection or infection when the animal was examined 48 hours after the bacterial inoculation.

3. Intradermal infiltration with normal rabbit serum did not protect against the injurious action of intradermally injected hemolytic streptococci in the doses used.

4. Antigenic relationships demonstrable by agglutination and agglutinin absorption tests were confirmed with regularity by *in vivo* local passive immunity experiments.

5. By this method of local passive immunity it is possible to differentiate a group of hemolytic streptococci causing erysipelas from a group of hemolytic streptococci responsible for scarlet fever, on the one hand, and on the other, from the large series of miscellaneous hemolytic streptococci producing a variety of pyogenic infections.

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Vital and Supravital Studies of the Cells of the Cerebrospinal Fluid and of the Meninges in Cats[1]

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I. Introduction

THE modern trend in histology and pathology has been towards the correlation of our knowledge of the connective tissues with the facts of hematology. In this sense histopathology today rests upon hematology; and it is to Ehrlich's fundamental analysis of the white blood cells that we must trace the beginnings of the modern period.

The next advance was Metchnikoff's demonstration in 1883 and 1892 of the importance of phagocytosis. He studied the phagocytic activity of the polymorphonuclear leucocyte and of those large mononuclear cells which can be seen in any inflamed tissue. He showed that the former ingested bacteria and contained a bacteriolytic ferment: while the latter engulfed and destroyed chiefly cellular debris. These latter cells he called "macrophages.

In 1902 Maximow (23) added to these differences the fact that the cells of inflammatory reactions may be divided into groups according to the speed with which they invade the injured area. The polymorphonuclear cells arrive within a few hours, the macrophages within a day, and the "fibroblasts" only after 24 hours.

Thus, by 1901, the existence of two lines of active cellular defense had been demonstrated, and a third reserve line of less phagocytic cells which were called "fibroblasts" because they were concerned presumably with tissue repair through the formation of connective-tissue fibers.

These facts form the foundations of our knowledge of simple inflammation. The many recent investigators have attempted to work out the details of this process. What are the sources of these cells? What is the relationship between the cells of an inflammatory reaction and those of the blood-stream or of the connective tissues? How many of the varied cell pictures which have been described and named in inflammatory processes represent distinct cell-strains;—distinct, that is, in origin, distinct in life-history, distinct in physiological, pathological, or immunological meaning? How independent are such cell-strains,—and how far may they be capable of transformation from one into another?

In these problems one group, led per-

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haps by Maximow, stands at one extreme: believing that the lymphocyte provides the body with a cell to which he has given the name of "polyblast," because he believes it capable of developing into any of the cells seen in inflammations. There are, on the other hand, many investigators, among whom we may instance Sabin and Cunningham, who have become convinced of the high degree of specificity of the several cell strains with which they deal. And there is a third group (among whom are Aschoff and Kiyono, and W. H. Lewis, and M. R. Lewis), who believe in the single identity of all of the highly phagocytic cells, whether they be found in blood stream or connective tissue; but whose position in regard to the less phagocytic cells has not as yet been made entirely clear.

It is quite generally conceded, now, that the study of fixed material alone cannot give evidence which is direct enough to solve these problems. The study of living tissues has therefore been an inevitable development, and has brought about the use of tissue cultures and of dyes which living cells can somehow incorporate into themselves in characteristic ways and with a minimum of injury.

Tissue cultures have been used to uncover the growth characteristics of isolated cell groups over periods of weeks and months.

The dyes have been used in two ways. They have been injected into living animals, and the tissues then examined at autopsy to determine the characteristic manner in which each tissue deals with the dye. This is spoken of as the method of "vital staining." It was first used for this purpose in 1906 by Bouffard (2) in France, and by Goldmann (16, 17, 18) in Germany. But Ehrlich's search for specific chemotherapeutic agents, and his study of the vital staining of nerve fibers with methylene blue laid the basis for these and all later workers.

The second way in which "vital dyes" are used has been called by Pappenheim (27) the method of "supra-vital staining," to distinguish it from the method of "vital staining." In this, tissues are taken fresh from an animal and the reactions of cells are then watched under the microscope as dyes are presented to them. The technique of supra-vital staining has reached its fullest development in the fundamental studies of Sabin and Cunningham: and it is their work which has made our own investigations possible.

All three of these methods have been used to some degree in the studies presented here. The meninges have been vitally stained by the injection of trypan blue into the subarachnoid space, thus producing a sterile inflammation, and picking out those cells which will ingest the particles of colloidal dye. The free cells of the spinal fluid have then been studied not only in fixed smears (prepared by a special method to be described below), but also by direct observation of the living cells in supra-vital preparations. Scrapings of living arachnoid cells have been studied in the same way, and have been allowed to grow in short-run tissue cultures. In addition, strips of formalin-fixed arachnoid and pia have been peeled from blocks of cord and brain by the method of Essick (12). And sections of all tissues have been cut. The technical details of each of

these methods are given in the next section.

By the use of these different techniques it has been possible to observe the pouring out of polymorphonuclear leucocytes from the rich vascular bed of the pia, and the rapid deterioration of these cells in the cerebrospinal fluid. It has been found that, as irritation of the meninges is sustained, lymphocytes become packed in the perivascular spaces; and that these lymphocytes begin to flood out of these perivascular channels into the subarachnoid space when the fluid is drained off, but can perhaps be more completely washed out by a simultaneous intravenous injection of hypotonic solutions. With the vital techniques it has been possible to distinguish both active and inactive phagocytes from lymphocytes and from the arachnoid lining cells; and evidence has been accumulated to show that these phagocytes are identical with the macrophages of the body in general, not transformed from other cells of the region, but arising from younger phagocytic forms in the meninges and vascular coats. And finally, the arachnoid lining cell has been seen to ally itself most closely with the great group of serosal cells of the body, and as with the serosal cell, a possible relationship to the fibroblast must be borne in mind rather than any intimate relationship to phagocytes.

It is a pleasure to acknowledge here our indebtedness to Miss Susan H. Wilkes for her excellent drawings of the living cells. Without them no graphic representation of these delicate appearances could have been presented.

II. METHODS

a. The production of meningeal irritation by injection of trypan blue into the cisterna magna

Puncture of the cisterna magna was performed according to the technique described by Wegeforth, Ayer and Essick (39). With a rigid needle, sharpened well to a short bevel, direct injury to the medulla occurred but rarely after some experience had been gained. We found an 18-gauge nickeloid lumbar-puncture needle most satisfactory, as did they. Our operations were all performed under ether anesthesia, and with sterilization of instruments, trypan blue, and skin.

The sample of cerebrospinal fluid for examination was collected in a small glass cannula, and the rest allowed to drain away. The trypan blue was thus injected into a subarachnoid and ventricular system that had been emptied as completely as possible. The fluid, of course, was warmed previously, and was injected quite slowly, the flow never being continued when any definite back-pressure was encountered. At the start, acute respiratory failure had been frequent—sometimes but not always responding to artificial respiration. It later became our practice to watch the cats closely during the first minutes after the injection, and if the breathing was at all light, without waiting for cyanosis to appear, the thorax was gently pressed in rhythm with the cat's own breathing until the animal was awake and out of danger. With these precautions, and with increasing proficiency, our acute operative mortality ceased.

It often happened, however, that several hours after a successful operation a cat died. Woollard (47) had a similar experience and felt that it was a question of the concentration of the dye. He dissolved his dye in Ringer's solution and found that solutions stronger than 0.1 per cent caused increased meningeal irritation and death. We found, however, that solutions as strong as 0.5 per cent and even 1.0 per cent could be injected safely if the dye was made up in distilled water. By running parallel groups of animals, it was found that the toxicity of the dye is greater in Ringer's solution than in distilled water, and that the toxicity of any given solution increases on standing. And on inspection of the solutions, it could be seen that the electrolytes of Ringer's solution partially "salt out" some of the colloidal dye, precipitating a thick mass in the bottom of the flask. A similar change appeared in a solution of dye in distilled water, if it was allowed to stand for several days.

It became clear, therefore, that one could reduce greatly this delayed mortality (1) by using a fresh solution of the dye in distilled water for each injection, and (2) by giving a weak initial injection of about 1.0 cc. of 0.25 per cent, and gradually working up to a dose of 1.0 to 3.0 cc. of 1.0 per cent dye. We found it possible to give as many as 5 injections in a week, and thus to produce rapidly a very high degree of irritation.

We preferred to inject small amounts of a relatively concentrated dye, not only to avoid acute pressure reactions, but also to minimize the leakage which always occurred to some extent through the puncture hole. Increased leakage with increased volume of injection was very evident at autopsy in the wider spreading of the blue through the fascial planes of the neck musculature and the deeper staining of the dorsal chain of cervical lymph glands. We wished to minimize this leakage in order to avoid any general stimulation of the blood-cell-forming organs of the body.

b. The dissection of the meninges

In 1920, Essick (12) published his studies of the response of the meninges to the stimulus of particulate matter. With his conclusion that the arachnoid lining cells themselves become transformed into the macrophages of the fluid and meninges, we have been forced to disagree. But his method of obtaining sheets of arachnoid tissue for study has proved invaluable to us.

From formalin-fixed material, a small block of cord or cortex with its membranes intact is pinned on sheets of cork under salt solution in a shallow dish. Under a binocular dissecting microscope the dura is then opened, great care being taken not to cut away the arachnoid at the same time. At the cut end of the block the arachnoid can then be picked up and, as gently as possible, with the finest iridectomy scissors, the arachnoid is slit down one side, retracted, and the trabeculae which bind it to the pia are severed. On to coverslips, which have been smeared with a little egg-albumen, flat spreads of arachnoid, with the trabeculae against the glass, can then be floated; and after drying can be dropped into stain. (We frequently used Mayer's carmalum, in order to secure a contrast with the trypan-blue inclusions of the macrophages.)

It was sometimes advantageous to increase the visibility of the membranes and trabeculae, before undertaking the dissection, by allowing the whole block to stain in carmine for an hour. Particularly in the more delicate tissues of normal cats and of young kittens was this helpful.

Where an uncomplicated view of a flat arachnoid sheet was desired, the trabeculae were cut away from the arachnoid as close to their points of attachment to it as possible. More often, however, it was desired to study particularly the fine trabecular strands themselves. For this purpose the trabeculae were severed close to their pial attachments, and were thus allowed to hang from the arachnoid sheet. With needles the piece of arachnoid was then torn gently into fragments, guiding the process on the cover-slip under the microscope. This exposed the delicate trabeculae in the interstices between the arachnoid fragments, which would otherwise have obscured them. This dissection must be done very gently. Rough handling can shake the large phagocytic cells loose from their points of attachment to the membranes.

Several attempts were made to secure preparations, similar to these, from the living animal, in order to study the living cells of the arachnoid in supra-vital preparations just as the living cells of the fluid were being studied. It was found, however, that better samples of living arachnoid tissue could be secured, and with greater technical ease, by merely scraping the under surface of the membrane with the edge of a fine straight knife. Flat plates of arachnoid lining cells and trabecular strands were thus secured. Some samples were studied on supra-vital films. Other scrapings were cultured in hanging-drop preparations in spinal fluid and in Locke-Lewis solution, and their reactions watched for several days.

Finally, of course, for the study of the vascular changes and the perivascular responses in the depths of the nervous system, sections were cut from various levels of the cord and also through the forebrain in such a way as to secure good samples of the choroid plexuses.

c. Brief review of former methods of studying the cells of the fluid and description of the methods used in these investigations

The study of the cells of the cerebrospinal fluid began with the work of Widal, Sicard, and Ravaut in 1901 (40). These observers developed a method which is usually spoken of as "the French method," but which has been displaced by improved techniques. They centrifuged samples of fluid at high speed for a long time and examined the sediment. The cells, when stained, were so degenerated that it was never possible to recognize any details more salient than the proportion of polymorphonuclear and mononuclear forms. Nor was this always easy. Their interest, therefore, was mainly confined to an estimation of the number of cells in the fluid from the number to be seen in a given number of fields.

In 1904 Fuchs and Rosenthal (15) introduced the modified counting-chamber which is still in use; and by using a combined staining and diluting fluid were able to recognize polymorphonuclear cells, lymphocytes, and

with fair accuracy the large mononuclear forms. Their main interest, however, was still directed towards variations in the number of cells to be seen.

It was not until Alzheimer (1) developed his method in 1907 that really satisfactory pictures of the cells could be secured. The great difficulty has always been that when a film of spinal fluid is spread on a coverslip and allowed to dry, as is done with a blood smear, the slow evaporation of the large amount of watery fluid in which the cells are suspended allows a marked shrinkage and degeneration of the cells to occur. The result is a very unsatisfactory cell stain. (This we found to be true even if the films are dried with heat.)

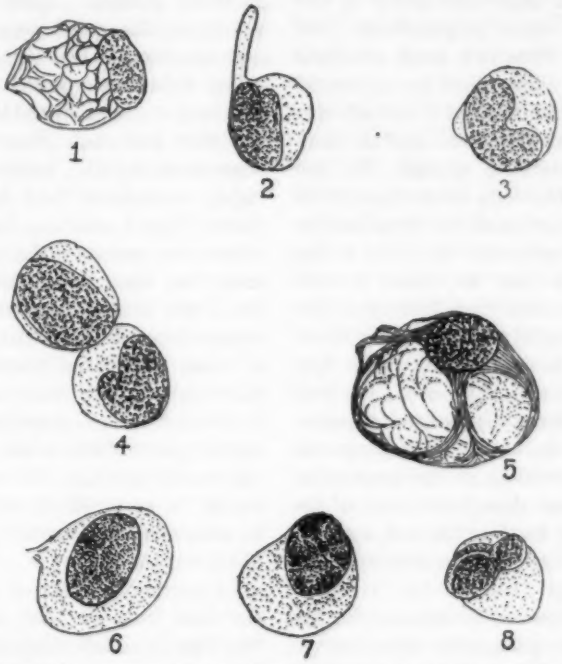
Alzheimer overcame this problem by centrifuging several cubic centimeters of fluid in the presence of a larger amount of strong alcohol, imbedding the coagulum, and cutting sections. This method yields beautiful cell stains: but it is time-consuming; sections from several layers of the coagulum must be studied, because the centrifugation alters the distribution of cells according to their specific gravities; and it does not make possible the separate examination of several samples of any one fluid (a procedure which came to have crucial importance in the course of these studies).

In this series of experiments, for our essential data, we have relied completely upon the study of living cells, in which the fluid may be examined drop by drop if desired. But for the sake of keeping some permanent record, and in order to correlate the study of living cells with the results of older methods of examination, we have developed a method for fixing the cells of the fluid which has proven quite satisfactory. This method was suggested to us by noting the success which Dr. W. H. Lewis has had in fixing tissue cultures with iodine vapour. In brief, our procedure is to seal a strip of glass to the bottom of a petri dish with a little balsam. A few crystals of iodine are scattered over the bottom of the dish which is warmed gently, just before using, until faint purple fumes fill the chamber. A coverslip, carrying a single large drop of fluid, is then placed on the strip of glass in the dish. The cells become fixed almost at once. The drop itself dries quite slowly; but when it is dry, the cells have settled down upon a small circular area of the slip, becoming firmly attached to the glass surface. The preparation is now deeply stained, with minute crystals of iodine and a fine film of coagulated protein overlying it. From this the excess iodine is washed out with a strong aqueous solution of potassium iodide, after which it is rinsed in water and stained. We used Mayer's carmalum to secure a contrast with the trypan blue inclusions, and the Unna-Pappenheim combination of pyronin and methyl-green for more detailed cell morphology. Sketches of cells fixed and stained by this method are given in Figs. 1 to 8.

The cell fixation secured in this way is excellent. Even very fragile cells, phagocytes swollen with vacuoles, or motile forms are perfectly preserved. Lymphocytes, which by the usual methods appear to have only round dense nuclei, with this fixation show as great a variety of nuclear shapes and chromatin patterns as in the living.

While the exact temperature is un-

important, high temperatures are unnecessary. Our routine was to place the petri dish in the corner of the warm box in which we were studying the living cells. Thus at 38° to 40°C. we white cells of the blood. Carefully cleaned slides are flooded with a solution of dye in absolute alcohol. This is drained off at once and the slide is then allowed to dry. The cells to



FIGS. 1 TO 8. Sketches of cells fixed by iodine vapour, deiodinised with KI, and stained. Figs. 1 to 4 and 6 to 8 are of cells from two normal cats. Fig. 5 is of a cell from an inflammatory exudate. Figs. 1 to 5 are carmine-stained.

(1) A highly vacuolated phagocyte. (2) An intermediate lymphocyte caught during active motility. The tail shows how the cytoplasm streams out behind the nucleus, and accounts for the so-called "tailed cells" of neuropathology. (3 and 4) Cells which may be either intermediate lymphocytes or small monocytes. (5) Enormous, balloon-shaped phagocyte. (6 to 8) Intermediate lymphocytes, at a slightly larger magnification, and stained in pyronin-methyl green. Two of them show clearly the perinuclear halo, the "clock-face" chromatin pattern, and the pink cytoplasm with a faint blue haze, of the so-called "plasma cell."

secured an adequate concentration of the iodine fumes, with a temperature that was itself normal for the cells.

For the study of the living cells we have applied to the cerebrospinal fluid the supra-vital method which Sabin (29, 31) developed for the study of the be examined are dropped upon this filmed surface, covered with thin coverslips, sealed with vaseline, and studied under the oil-immersion lens in a hot box. The details of the technique are presented fully in the papers of Sabin (31), of Cunningham (8), and

of Sabin, Cunningham and Doan (33) referred to below. In only one point did our procedure differ from theirs: we found it necessary to weaken the strength of the dyes.

This is an important factor in the success of such preparations. For making the films two stock solutions are kept on hand: these are saturated solutions of neutral red (Grubler) and of janus-green (National Aniline Company) in absolute alcohol. In the spinal fluid the cells are so susceptible to the toxic action of the dyes that for the dilute solutions with which the slides are flooded we found it best never to use more than 20 drops of the stock solution of neutral red to 10 cc. of absolute alcohol for the single dye, and for the double dye never more than 3 drops of the stock solution of janus-green in 5 cc. of the diluted neutral red. Any staining of the nucleus by either of these dyes is evidence of the approaching death of the cell; and if it occurs rapidly, it means that the stain is too strong.

The neutral red is essential for the study of the phagocytic potentialities of the cells: the janus-green for their mitochondria. The green is more toxic than the red dye and is used more sparingly. It seems to have an inhibiting effect upon motility, even when it is not strong enough to stain the nuclei. The motility of cells, therefore, cannot be used as a differential criterion in its presence.

III. THE CELLS OF THE CEREBROSPINAL FLUID

a. Terminology

The physical and chemical properties of the cerebrospinal fluid differ so profoundly from those of blood plasma, that it was to be expected that in our supra-vital films the cells in suspension in cerebrospinal fluid should exhibit slight differences from the same cells in blood plasma. These differences are three: the cells, especially polymorphonuclear neutrophilic leucocytes, deteriorate more rapidly; all cells tend to round up and lose motility; and they lose their phagocytic inclusions more rapidly, leaving behind a highly vacuolated and foamy cytoplasm (Figs. 1 and 5). Despite these differences, however, the essential criteria that have been worked out on blood are directly applicable to the cells as found in the fluid. The details of these differential points are given below in the appropriate sections. It is interesting to consider that the cerebrospinal fluid, which is the environment in which the neuron lives, should be so strikingly deleterious in its effects upon the white cells of the blood-stream.

In supra-vital films of the cells of the fluid from our cats we have distinguished the following cells:

(1) Polymorphonuclear neutrophilic leucytes.
(2) Polymorphonuclear eosinophilic leucytes (rarely).
(3) Lymphocytes: Small, intermediate and large (the last rarely).
(4) Phagocytes or "Macrophages." (a) monocytes; (b) clasmatocytes.
(5) The arachnoid lining cell itself (rarely).

We would emphasize here our efforts to bring our terminology into harmony with that of modern studies of other mesodermal tissues. Neuropathology has suffered from its isolation from general pathology; and one of the re-

sults has been a tendency to look upon every cell picture as representing a cell-strain and every such cell-strain as meriting a new name. In contrast to this, since we have found no cell-strain in our studies which is peculiar to this region, we felt justified in using only those names which emphasize the identity of these cells with those of the connective tissues generally.

We have used the term "macrophage" or "phagocyte" interchangeably to designate the whole group of vigorously phagocytic cells. Their subdivision into "monocyte" and "clasmatocyte" rests upon morphological and developmental evidence which is presented in full in the appropriate section. The former name is reserved for that phagocyte which is a normal inhabitant of the blood stream, and the latter for the "wandering endothelial phagocyte" of Sabin *et al.* (33). We have found no need to resort to such special names as *Gitterzellen*, or *Kornchenzellen*,—all of these appearing merely as special phases of the phagocytes in activity. And the so-called "plasma cell" of neuropathology has seemed to us to be identical with the intermediate lymphocyte of Sabin (31),—as is discussed below.

Our terminology in general is that of Sabin and Cunningham. Since we were using the differentiating criteria which they have worked out (Sabin (31), Sabin *et al.* (33), Cunningham (6, 7), Cunningham *et al.* (10)), not to make use of the terminology which they have found most useful would obviously have involved us in a constant labor of translation from one set of names to another.

b. The "effective dose" of trypan blue

We found no dose of trypan blue so small that it failed to cause an outpouring of cells. This is not in accord with Woollard's experience (41). Woollard claimed that with injections of several cubic centimeters of 0.1 per cent of the dye dissolved in Ringer's solution no pleocytosis occurred in the fluid. We did not try 0.1 per cent of the dye in Ringer's solution, but used this same strength in distilled water, a much less toxic and less irritating procedure. The outpouring of polymorphonuclear leucocytes in response to even 1 cc. of this occurred invariably, but within 24 hours had passed its peak and by 48 hours had almost cleared.

We attribute the difference between our experience and that of Woollard to two things: first, to the greater ease of recognizing cells by the supravital method of studying the fluid (a method which was only in its incipient stages of development when Woollard made his investigations and which he used only when he injected laked red cells in addition to the dye); and secondly, to the transient character of the exudate obtained with such weak doses. Woollard made his examinations of dye-injected cats only on the second day, and with the methods then at his disposal could easily have overlooked the slight residual pleocytosis at the time. Certainly, this would account for his missing the pleocytosis that occurred in response to the initial injection, at any rate;—how to account for his failure to observe it as repeated injections increased the irritation is not so clear.

c. The course of the reaction to injections of trypan blue into the cisterna.

With the hope of tracing the exact course of the inflammatory responses of the meninges, hundreds of differential counts were made with the supravital films. Unfortunately, it was only towards the end of these counts that it was discovered that the proportions of the different cells vary with different fractions of a fluid. (The detailed evidence on this point is presented in the section on lymphocytes.) It then became evident that to add anything in this direction to our existing knowledge of meningeal responses, differential counts must be made upon the first few drops and the last few drops of cerebrospinal fluid at every stage of the reaction. It is only in this way that we can determine exactly how the polynuclear response gives way to a mononuclear response, and how the mononuclear elements balance between phagocytic and lymphocytic forms. Such studies are slow in accumulating and cannot be presented here. For this reason we are presenting in the following sections only those counts which brought to our attention the differences between various fractions of a fluid (Tables I and II), and a general summary of the rôle of each cell in the inflammatory reaction as we produced it.

IV. THE POLYMORPHONUCLEAR NEUTROPHILIC LEUCOCYTE IN THE CEREBROSPINAL FLUID

a. Characteristics in supra-vital films

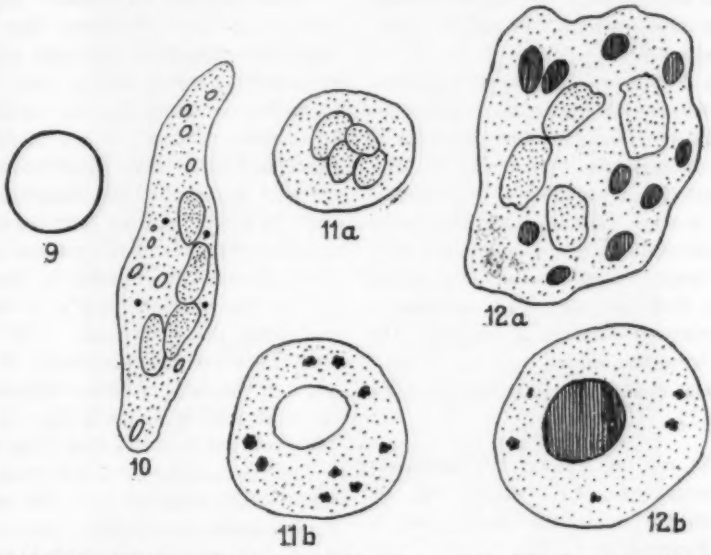
In two respects, the polymorphonuclear neutrophilic leucocyte in a supra-vital film of cerebrospinal fluid differs from the same cell in a supravital film of blood. In the fluid, the cell degenerates rapidly, so that in about two hours many of these cells could not be recognized by one who had not followed the steps of the degenerative process, whereas in an uninjured blood film degenerating forms occur only after several hours. And similarly, active motility is rarely seen in the fluid except when the fluid is turbid with cells and protein during the height of an acute inflammation, and even then persists for only a few minutes. In a blood film, on the other hand, a majority of these cells are motile, and the motility persists for a long time. The motile polymorphonuclear leucocyte presents the same appearance, whether it be in blood or fluid. The non-motile and degenerative phases in the fluid are a little different from those seen in the blood.

In its active phase (Fig. 10), the cell is the most rapidly moving object to be seen. The cytoplasm streams around, over, and ahead-of the many-lobed nucleus, pushing out pseudopodia of all shapes. The cell sometimes rounds-up, the cytoplasm circling more slowly around the nucleus. This may presage the transition into a permanently non-motile phase, or may change again to active moving. In the cat, the cytoplasmic granules of the motile cell are so faint as to be barely visible; and, in contrast to those in certain other animals, fail to stain with neutral red. The cell is weakly phagocytic for the red dye, however, developing in the course of time a few small pale vacuoles scattered through the cytoplasm. With janus-green, a few blue-green mitochondria can be made out.

The non-motile cell in the fluid must be recognized as having two special forms of its own. In a freshly made film, especially if the film is made from a large drop of fluid so that the cells float freely between the coverslip and slide, the leucocyte is small—just larger than a lymphocyte (Fig. 11a). It is highly refractile, and perfectly round. Its mutilobed nucleus is centrally placed and clearly visible, however, so that the cell can never be mistaken for a lymphocyte. This cell takes up no dye of any kind.

Then, as this cell settles on to the slide, it forms the second type of non-motile cell (Fig. 12a). It spreads out into a very large, irregular shape, so pale that unless the light be diminished sharply it can easily be overlooked. In this cell abundant large vacuoles of neutral red may gradually appear, scattered through the pale cytoplasm; and here, were it not for the characteristic fragmented nucleus, the abundance of dye-filled vacuoles could make differentiation from a stimulated macrophage quite difficult.

In both of these forms, as degeneration sets in, the nucleus seems to flow together to form one large central globule. This may or may not be-



FIGS. 9 TO 12. Sketches of the polymorphonuclear neutrophilic leucocyte of the cerebrospinal fluid in supravital films.

(9) An erythrocyte drawn as a size index. (10) The actively motile cell. The tiny droplets represent pink vacuoles of neutral red; the heavy dots indicate mitochondria. (11a) The small, round, non-motile phase, without any dye. (11b) The degeneration of 11a with the formation of one large central vacuole instead of the nucleus, and the appearance of a few coarse granular masses in the cytoplasm. (12a) The spread-out non-motile phase, with a fragmented nucleus and large vacuoles of dye. (12b) The degeneration of 12a, which differs from 11b only in that the dye has stained the central vacuole deeply.

come deeply stained,—depending apparently upon whether the degeneration has started in the first or second type of non-motile cell. Finally, all that can be seen is this central globule which is either perfectly structureless and translucent or else garnet-red, and around it a circle of equally clear cytoplasm with a few large, highly refractile bodies scattered through it (Figs. 11b and 12b).

We do not feel entirely certain that this picture of a degenerated cell comes exclusively from the polymorphonuclear neutrophilic leucocyte. It may be that the end stage of a lymphocyte or of a small, unstimulated phagocyte is quite similar to it. Whenever such cells began to appear in our preparations, therefore, all efforts to make a quantitative differential count of the cells were terminated.

These cells are all illustrated in Figs. 10 to 12.

b. The rôle of the polymorphonuclear leucocyte in the reaction of the meninges to subarachnoid injections of trypan blue

The significance of the leucocyte reaction to our non-infectious inflammations is obscure. Confirming Essick (12), we found that it was the first cell to be poured out,—appearing in great numbers within a few hours, reaching a very high peak within twenty-four hours, and then rapidly dropping off, until by the third day the other cells begin to outnumber it.

Neither in our vital films, nor in carmine-stained smears of the cells of the fluid, nor in peeled-off strips of inflamed arachnoid have any leucocytes been seen containing recognizable particles of trypan blue. This is in harmony with the findings of M. R. Lewis (20) in cultures of drops of blood.

It is assumed that polymorphonuclear leucocytes invade an injured region by adhering to the endothelium of dilated vessels where the stream is slow, and there crawling through the walls and out into the tissues. It puzzled us at first, therefore, that the relatively avascular arachnoid should so rapidly become dotted over with these leucocytes during the height of an acute reaction to an injection. Material from animals killed before the reaction has reached its height shows that the leuccytes arise from the many vascular channels of the pia and must reach the arachnoid partly by floating to it in the fluid and partly by crawling along the trabeculae. This contrast between the vascularity of the pia and the lack of blood channels in the arachnoid is shown in Figs. 13 and 14 from a cat injected with India ink.

The mechanism by which these leucocytes are removed from the spinal fluid remains uncertain. Their rapid degeneration in supra-vital fluid preparations would suggest an equally rapid degeneration in the body. In the freshly made film non-motile forms abound, but it is not possible to say with certainty that the debris occasionally seen in such films has arisen from the breaking up of these non-motile forms.

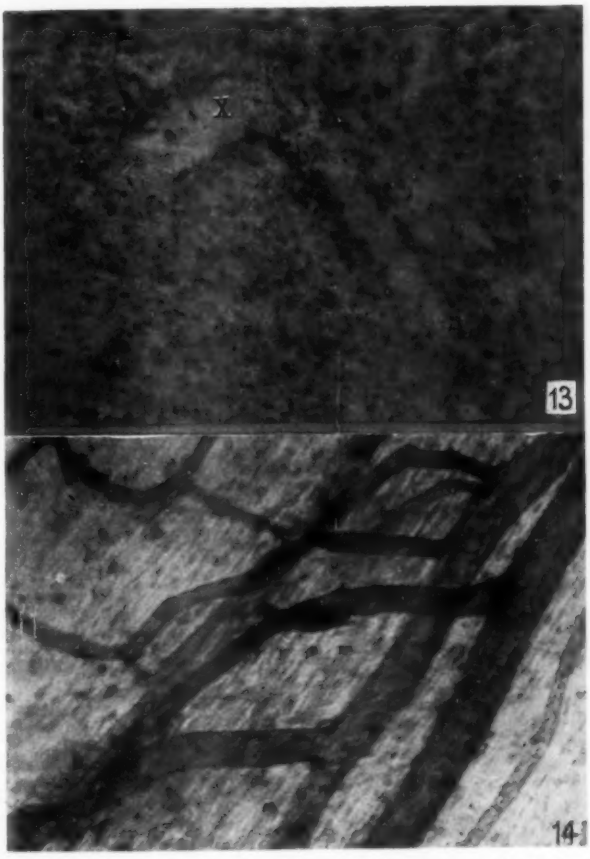
V. THE POLYMORPHONUCLEAR EOSINOPHILIC LEUCOCYTE

This cell is as readily recognizable on its rare appearance in the fluid as in the blood, as characteristic in supra-vital films as in the more familiar, stained blood-smears.

In a supra-vital film of blood the cell is almost as actively motile as the neutrophilic leucocyte; but in the fluid we have never seen it show any motility.

The characteristic granules are large, slightly ovoid, brilliantly yellow-red with the neutral-red dye, and all of one size and color.

This cell appeared almost as an accident in our experiments. It never took part in the initial outpouring of neutrophilic leucocytes. It seemed to come exclusively after the trauma of



FIGS. 13 AND 14. Photographs of meningeal spreads from a normal cat after an injection of india ink through the aorta. Formalin. Carmine. × 150.

(13) Arachnoid from the dorsal surface of the cervical region, showing the absence of blood vessels. X indicates the point at which two vessels from the pia touched the arachnoid but were peeled out in the dissection. (14) Pia that was exactly subjacent to the area of arachnoid shown in 13. The ink-filled arteries and veins form an abundant meshwork, which surrounds the cord and penetrates it from all surfaces.

some previous puncture had caused a little bleeding into the fluid, after which it may constitute as much as 15 per cent of the polynuclear forms.

Unlike the neutrophilic leucocyte it was quite persistent in the supra-vital films of fluid and no degenerating forms were encountered; which may explain the high percentages sometimes found.

VI. THE LYMPHOCYTES OF THE SPINAL FLUID AND THE SO-CALLED "PLASMA CELL"

a. Characteristics of lymphocytes in supra-vital films

With the introduction of the supra-vital technique there has been recognized not merely two forms of the lymphocyte but three,—a "large," an "intermediate," and a "small" (Sabin, 31). The size differences are made up in some degree by an increase in the size of the nucleus, but even more by the increasing amount of the surrounding cytoplasm. The small cell has a narrow, often barely visible rim of cytoplasm: the large cell has abundant margins. The small cell is just larger than a red cell: the intermediate is a little smaller than the round non-motile leucocyte; and the large cell is definitely larger than such a leucocyte.

In their most recent studies (34) Sabin, Cunningham, Doan and Kindwall report that at 40°C. all three of these forms have been seen to show motility. The large cell shows this least often, however, and it is only the intermediate form which exhibits vigorous motility frequently, and at body temperature. This is probably an age difference. They are led to the conclusion that the large lymphocyte with its abundant mitochondria is the youngest adult form; and that the intermediate cell, with its heightened motility and its diminishing but still ample mitochondria, corresponds to the intermediate age; while the small cell, with its lessened motility and its disappearing mitochondria, is an aging cell. The occasional small lymphocyte seen without any mitochondria is thought to be actually senile and verging on degeneration.

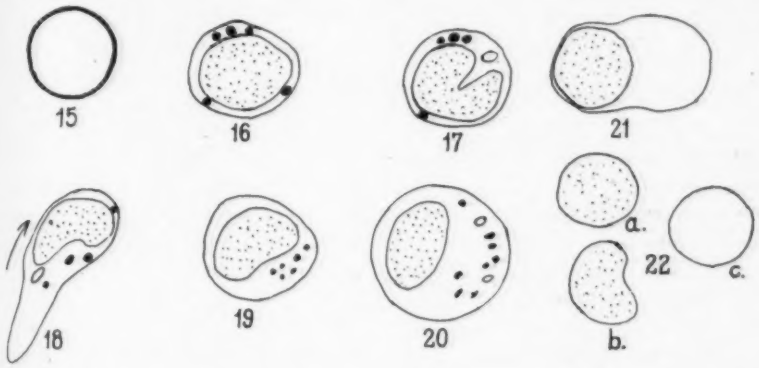
Motility of any lymphocytes is of course seen but rarely in the films of cerebrospinal fluid. Whenever seen, however, it is the same peculiar type of motility as that shown by lymphocytes in blood films. The cell moves by a slow streaming of the cytoplasm as a whole, putting out no pseudopodia, and always pushing its nucleus along in front of it. The entire outline of both the nucleus and the cell body is made irregular by this activity. In the spinal-fluid, we have observed motility only in the intermediate lymphocytes (Figs. 15–22).

The nucleus of living lymphocytes is rarely perfectly round or perfectly oval. Even in the small lymphocyte, in which a round nucleus occurs more frequently than in the larger forms, a tendency to indentation is usually visible at some point: while in the larger cells an eccentrically placed, somewhat kidney-shaped nucleus is the rule. Furthermore, nuclei have actually been watched while changing their outlines from oval or round to indented forms, and the reverse. This explains the difficulty that has always been experienced by those who have tried to differentiate the larger lymphocytes from the other mononuclear cells of the blood stream and of the

connective tissues, on the basis of their morphology in fixed preparations. In the supra-vital films, fundamental differences in the responses of the cells to vital dyes make the differentiation more exact:

(*a*) The lymphocytic strain has but little tendency to phagocytize neutral red. More than two or three tiny droplets are but rately seen. Despite prolonged irritation with trypan blue of janus-green, they take on a characteristic blue-green color. In all other mature cells, whatever mitochondria are present, are small and scattered. In lymphocytes these bodies tend to appear as large dots or rods clumped in the cytoplasm opposite the nucleus.

The lymphocyte undergoes two types of degeneration in supra-vital films. In the first place, it is sensitive to dyes,



FIGS. 15 TO 22. Sketches of the lymphocytes from supra-vital films of cerebrospinal fluid.

(15) Erythrocyte for comparison. (16, 17) Small lymphocytes with mitochondria (solid dots) and one vacuole of neutral red (tiny circle). (18, 19) Intermediate lymphocytes;—18 is in active motion in the direction of the arrow. (20) Large lymphocyte. (21) The beginning of lymphocytic degeneration by the extrusion of the nucleus. (22) *a* and *b* are naked lymphocytic nuclei; *c* is an empty bubble of cytoplasm.

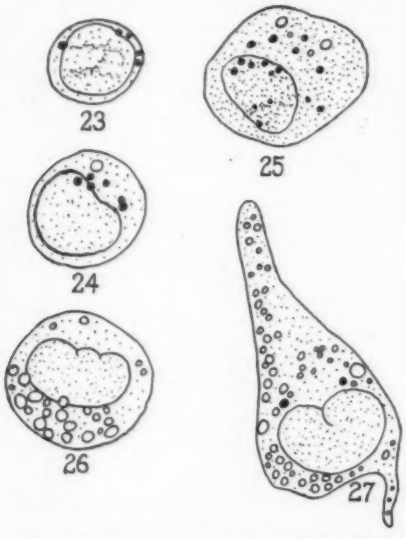
injections, we have never seen as much as that pictured in Fig. 50 of Cunningham, Sabin and Doan (10). The phagocytic tendency of the cells is thus seen to be very limited.

(*b*) The whole lymphocytic group is sharply characterized by the presence of prominent mitochondria. These bodies are so large and refractile that they can often be recognized even when unstained: but in the presence especially to janus-green, and a staining of the nucleus is often seen before any other cell shows this toxic effect. But secondly, even in the absence of dye effects, an extrusion of the nucleus has been observed to take place. And naked lymphocytic nuclei have been seen even in fresh films, as though the same thing had occurred in the body.

The lymphocytic strain is illustrated in Figs. 15 to 22.

b. The lymphocyte of the normal fluid and in inflammatory responses to subarachnoid injections of trypan blue into cats.

The lymphocyte is a normal inhabitant of the spinal fluid in cats. It does not, however, appear to be the sole normal cellular element to be found. Immature forms of phagocytes have also been seen in animals in whom we had no evidence of central nervous system disease (Figs. 23–27). (This point is discussed a little more fully in the section on the phagocytes of the fluid.)



FIGS. 23 TO 27. Sketches of lymphocytes and phagocytes from cerebrospinal fluid of a normal cat.

(23) Small lymphocyte. (24) Intermediate lymphocyte. (25) Large lymphocyte. (26, 27) Monocytes.

The rôle of the lymphocyte in pathological processes of the meninges and nervous system has been described in many ways. The recent tendency has been to associate it most intimately with certain diseases; poliomyelitis, lethargic encephalitis, tuberculous meningitis, lues,—and with chronic processes in general. There have been descriptions of outbreaks of benign lymphocytic meningitis in children, obscure in origin. Most of these reports are based upon counting-chamber differentials, however,—and one must turn to the older literature for painstaking applications of Alzheimer's method for dependable data—Henderson and Muirhead (19); Cotton and Ayer (13); and others.

We did not expect to find the lymphocyte playing a very important rôle in our short-run irritations. And in fact, on only six occasions, in our first one hundred counts on thirty cats, did we find a predominant lymphocytosis. Six cats, each on only one occasion,

inexplicably threw off a great number of lymphocytes. For a long time we had no explanation for this, but noted that each of these counts was made in a fluid that was contaminated with blood. This later proved to be a helpful point (see Table I).

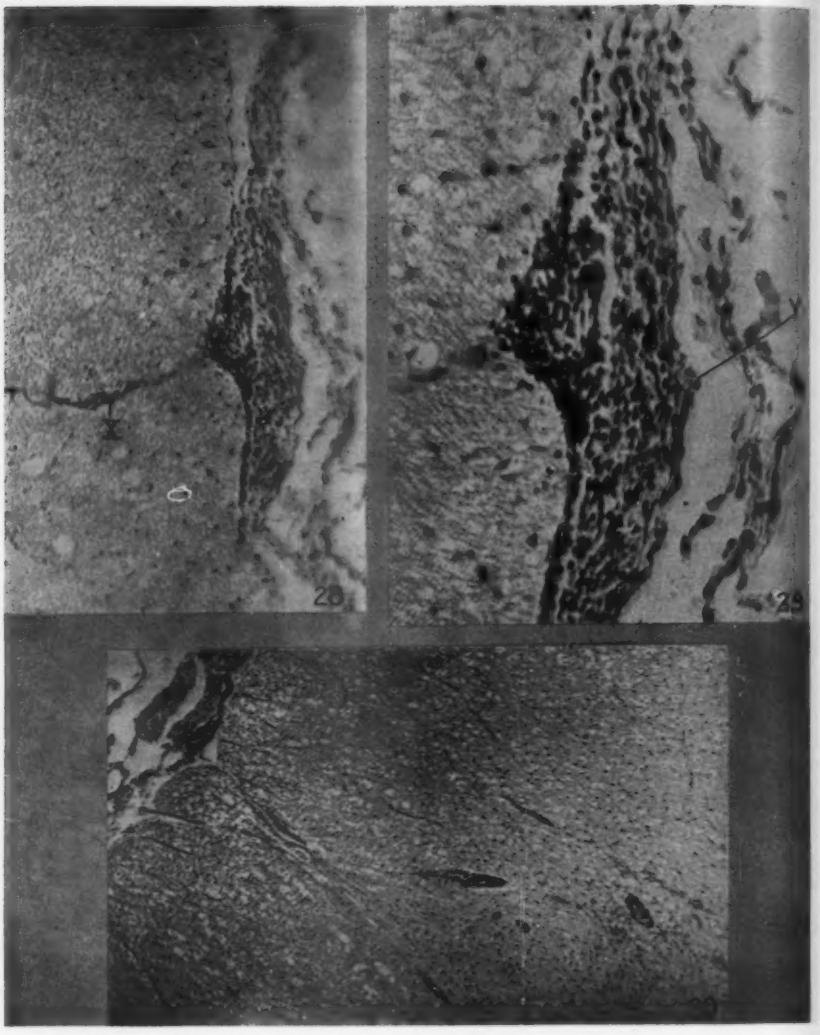
Sections from the spinal cords of some of these thirty animals,—cats in which no predominance of lymphocytic forms had ever been observed in the fluid, as well as those in which the unexplained lymphocytosis had occurred,—showed a striking infiltration of the peri-vascular channels with small mononuclear cells which were indistinguishable in fixed tissues from lymphocytes (see Figs. 28–31). This suggested to us the possibility that under certain conditions these cells might be poured out from the peri-vascular channels into the subarachnoid space, and thus account for these six mystifying lymphocytic reactions.

To test out this hypothesis, we made differential counts upon the first drop that appeared in the needle and upon nearly the last drop that could be secured.[2] Without an exception, in every such experiment, we found that the percentage of lymphocytes increased markedly in the "last drop" sample. The accompanying table (Table II) gives a group of typical

TABLE I

	POLYMORPHONUCLEARS	LYMPHOCYTES	MONOCYTES	CLASMATOCYTES
	per cent	*per cent*	*per cent*	*per cent*
Cat 5. Count made 2 days after an injection of laked blood from another cat. The fluid was contaminated with fresh blood	21	77	2	
Cat 6. Count made on bloody fluid two days after an injection of trypan blue	35	63	2	
Cat 11. Count made on blood-contaminated fluid, two days after an injection of laked blood and trypan blue	20	68	2	8
The following day another fluid yielded	48	40	8	4
All later acute reactions in this cat were predominantly polymorphonuclear; *e.g.*, 3 days later,—2 days after a fresh injection of trypan blue—a clear fluid showed	74	1	6	2
Cat 14. On the day following an initial injection of trypan blue, bloody fluid was counted	10	60	8	12
Cat 26. Five injections of trypan blue were made into this cat in 5 days. During the course of these injections two counts had shown	88.5	1	8.5	2
the following	42	10	38	4
2 days after the last injection, however, a fluid contaminated with blood yielded	20	45	29	6

[2] There is, of course, no "last drop," since the elaboration of new fluid will maintain a slow flow for several hours. This flow, after 2–4 cc. have drained off in 3 to 5 minutes, drops to a rate of 1 to 2 drops per minute. It was when the drainage approached this rate that we took our "last drop" sample.



FIGS. 28 TO 30. Figs. 28 and 29, photographs of sections of the cord of Cat 12, which had received repeated injections of trypan blue. The cat was killed after drainage of the subarachnoid space, by the injection of saline followed by formalin through the aorta. Stained with carmine. Fig. 28, (× 140) shows a perivascular channel at X filled with cells, and out in the subarachnoid space over the mouth of the perivascular channel lies a heap of lymphocytes, on the outer margin of which are the blue-filled phagocytes. It is an inescapable conclusion that the lymphocytes have been extruded from the perivascular channel. (29) The same thing (× 290). Even without the color contrast of the section itself, the zone of phagocytes on the edge of the heap of lymphocytes is visible at Y.

Fig. 30: Perivascular lymphocytic infiltrations in the cord of Cat 20, after repeated injections of trypan blue. (× 78.)

TABLE II

(Only those counts are presented here in which enough cells were found to make the percentages significant—*i.e.*, 50 to 200)

	POLYMORPHONUCLEARS	LYMPHOCYTES	MONOCYTES	CLASMATOCYTES
	per cent	*per cent*	*per cent*	*per cent*
Cat 34 received 6 injections of trypan blue in 14 days.				
Count 1— made 2 days after the 6th injection:				
First drop	30	20	40	10
Last drop	0	80	16	4
A seventh injection of blue was then given, and 12 days later Count 2 was made:				
First drop	21	37	34	6
Last drop	13	48	11	11
An intravenous infusion of distilled water was then begun:				
After 10 minutes	6	72	4	4
After 20 minutes	12	80	4	—
Cat 35 received 6 injections of trypan blue in 14 days.				
Count 1, made 5 days after last injection:				
First drop	22	15	51	6
Last drop	1	71	15	13
A 7th injection was given; and 7 days later Count 2 was made:				
First drop	80	1.5	18	0
Last drop	18	36	4	12
An 8th injection was given. And 2 days later the final counts were made:				
First drop	45	20	20	3
Last drop	18	38	8	30*
Cat 38 received one injection.				
Count was made three days later:				
First drop	70	8	14	8
Last drop		16	6	0
Cat 41 received 3 injections of blue in 3 days.				
3 days later the count was made:				
First drop	18	2	56	16
Last drop	24	40	8	20
Cat 42 received 3 injections of blue in 3 days.				
3 days later the count was made:				
First drop	42	4	28	16
Last drop	4	80	8	0
A very slow intravenous infusion of 0.6 per cent saline was then started.				
After 20 minutes	0	100	0	0
After 40 minutes	0	100	0	0
Cat 50 received 4 injections in 5 days.				
Count was made 3 days after the 4th injection:				
First drop	48	10	22	16
Last drop	16	66	4	10

* This was an exceptional cat who threw off great sheets of clasmatocytes such as no other cat in our series showed.

results. This explains the six lymphocytic reactions mentioned above: It had been our practice to take our sample for counting from the first drops that appeared as the stylette was withdrawn. These six mystifying fluids, however, had been contaminated with blood; and we had therefore allowed them to drain until they had become as clear as we could get them,—with the result that these six counts alone (out of that whole group) had been made upon "last drop" samples. This accounted for their high lymphocyte counts.

We next carried our experiment one step further. Weed has shown that the intravenous administration of hypotonic solutions not only raises intracranial pressure (Weed and McKibben, 38), but that it also distends the perivascular channels of the central nervous system (Weed, 37). Acting upon this hint, we performed the following experiments. Four cats were subjected to repeated injections of trypan blue, until a fairly chronic stage of irritation had been reached. First and last drop differentials were then made, and after this a slow intravenous infusion of distilled water or 0.5 per cent saline was begun. This would start up a renewed flow of fluid at once, and after this had gone on for some minutes one or more further samples were taken and counted. Here again the results were consistent for all the experiments, and in each case the percentage of lymphocytes rose even higher after the infusion was begun than in the last drop which had appeared spontaneously. We present an abstract of the records of one such experiment:

Cat 34 received 7 injections of dye in 15 days.

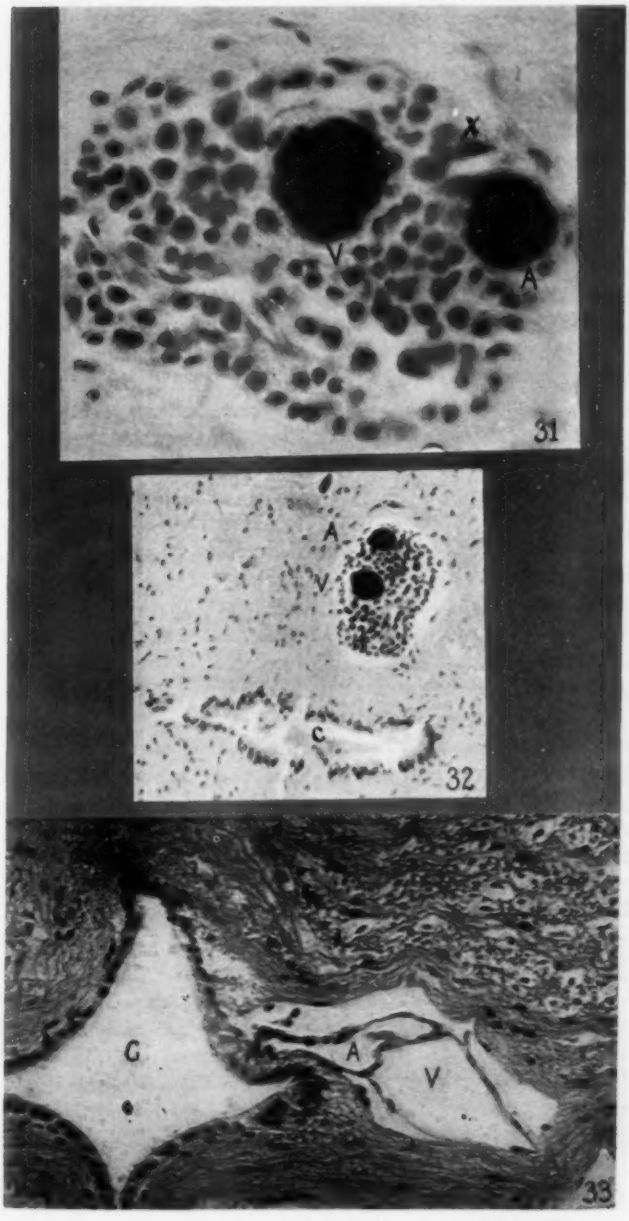
Shortly after the sixth injection, a count was made on the first drop, and on the last drop obtainable by spontaneous drainage. Between the first and last drops the polymorphonuclear neutrophilic leucocytes dropped from 30 per cent to 0, the macrophages dropped from 50 to 20 per cent, and the lymphocytes rose from 20 to 80 per cent.

The seventh injection was then given, and the reaction allowed to subside for several days, after which the following counts were made:

	FIRST DROP	LAST DROP
	per cent	*per cent*
Lymphocytes	37	48
Monocytes	34	11
Clasmatocytes	6	11

FIGS. 31 TO 33. Fig. 31 (× 680) and Fig. 32 (× 170), photographs of the central canal "C", the adjacent artery "A", and vein "V", and the perivascular channel choked with cells. The artery and vein are ink-filled from a post-mortem vascular injection. The cat (Cat 26) had been repeatedly injected with trypan blue. The cells which choke the perivascular channel are all mononuclear forms, showing the entire size-range of the lymphocytic series. Many of these lymphocytes show the eccentric nucleus and the clock-face chromatin pattern of the so-called "plasma cell," even in the photograph. The only blue-filled phagocyte to be seen lies along the outer wall of the channel, at X.

Fig. 33: Central canal, artery, vein, and perivascular channel from Cat 34 (× 245). This cat had been even more heavily irritated than Cat 26. Counts of the free cells of the fluid showed a heavy preponderance of lymphocytes in the last-drop samples (see protocol in text). The cat was drained and infused with a hypotonic solution intravenously before death. The photograph shows the distended canal, and the perivascular channel emptied of all but 3 cells.



FIGS. 31 TO 33

An intravenous infusion of distilled water was then begun, the renewed outpouring of spinal fluid beginning almost at once. After a few minutes a third and then a fourth drop were examined and showed:

	THIRD DROP	FOURTH DROP
	per cent	*per cent*
Lymphocytes	72	80
Monocytes	4	4
Clasmatocytes	4	0

Our preliminary studies of the tissues of animals which have been treated in this way show a striking washing out of the perivascular infiltration by the infusion and drainage. The possible therapeutic value of this procedure in diseases in which there is reason to believe that the perivascular channels are the sites of part at least of the inflammatory process is obvious. This aspect of these results is being studied further.

Perivascular infiltrations and the results of the intravenous infusions and of cisterna drainage are pictured in Figs. 28 to 33.

c. The so-called "plasma cell"

The application of Alzheimer's method of studying the cells of the spinal fluid revealed the presence of a cell which had certain constant morphological characteristics when stained with the Unna-Pappenheim stain of pyronin and methyl-green. Moreover, this cell was recognized not only among the free cells of the fluid but also in sections of the nervous system in the perivascular channels. It was called a "plasma cell," although its identity with the "plasma cell" of general pathology has never been completely established.

The cell is described as varying in shape from round to somewhat irregularly elongated. It is usually larger than a small lymphocyte. The nucleus is often eccentric and is surrounded by a halo of pale cytoplasm, beyond which the rest of the cytoplasm is more deeply stained. Although the cytoplasm is supposed to have an almost specific reaction to pyronin, there is also, according to Unna, a basophilic granulation too fine to be seen clearly. And in the nucleus the chromatin is characterized by a pattern which has been called "clock-faced," because of the radial arrangement of wedge-shaped masses with their broad bases pointing out and with pale radiating spokes between (see Figs. 6, 7 and 8).

There was some early dispute as to the existence of such a cell in the fluid, due to the faulty technique then in use. Nissl first doubted and later accepted it. All the more recent workers have recognized it (Turner, 36) and have concentrated their efforts on an attempt to show its relationship to the lymphocyte. Just what that relationship is has not been clearly defined,—but many texts picture cells which are pointed out as "transition cells" between the lymphocyte and the plasma cell (Spielmeyer, 35; Plaut *et al.*, 28; Cotton and Ayer, 3; Henderson and Muirhead (19). Councilman (4) even recognized its amoeboid nature. It is some times looked upon as a pathological or degenerating lymphocyte.

We believe that, by the supra-vital methods of observation, it is possible to recognize that the so-called "plasma cell" actually is a lymphocyte,—the "intermediate lymphocyte" of Sabin's classification. The characteristics of the cell are all of importance in this

connection. It is intermediate in size between the small and the large. It is capable of vigorous motility; and yet in any preparation not all of the intermediate lymphocytes are motile, many being rounded and still. But when in motion, it moves slowly across the field, pushing its nucleus ahead of it, drawing its cytoplasm slowly after, and sometimes leaving an area of thinner cytoplasm around the nucleus (Figs. 18, 19). By these characteristics of the intermediate lymphocyte, the oval or elongated and sometimes irregular shapes of the plasma cell can be understood,—as well as the perinuclear pallor sometimes seen.

Nor is the chromatin pattern of the nucleus hard to understand on this basis. Sabin's evidence (34) points to the likelihood that the motile or "intermediate lymphocyte" is the cell in its prime, and that the small lymphocyte is a somewhat shrunken and aging form. Naturally, every transition can be seen between them. And equally naturally, with this general decrease in size, the nucleus becomes more compact and more densely stained and loses, step-by-step, the open "clock-face" appearance that can at times be seen in the fixed preparations of the larger cells.

In iodinized drops of fluid these intermediate lymphocytes are indistinguishable from plasma cells—and in vital films there are no "plasma cells," unless they be the intermediate lymphocytes. We believe that the two, are one,—and that they are a *normal finding* in the spinal fluid of cats.

That the small, aged lymphocyte is more often seen in the fluid of normal cats is quite understandable. And in pathological states in which lymphocytes are increased it is inevitable that the young adult—the intermediate lymphocyte—should increase in numbers. This is just what is seen in the counts of "last drops" in which the proportion of intermediate lymphocytes rises sharply. And it is also what is seen in paresis, for instance, in which plasma cells are so abundant that they were thought for some time to be almost pathognomonic of the disease. They suggest, of course, only a lymphocytic increase, and not any special disease process.

VII. THE PHAGOCYTES OF THE SPINAL FLUID AND MENINGES

a. Recognition of both active and inactive phagocytes by the use of vital methods

With any technique, the phagocytic nature of a cell is recognizable at a glance after it has once ingested bits of debris or fat, pigment or globules of dye. In fixed material, even if some reagent through which the tissue has passed has dissolved out the ingested matter, still the foamy and swollen cytoplasm, the emptied vacuoles, the small nucleus crowded to one edge of the cell, all make the interpretation easy. The phagocytic history of the cell is read from its appearance (Figs. 1, 5).

To recognize as potentially phagocytic a cell which has ingested no foreign particles is far more difficult. Indeed, it is impossible to do so with any certainty in material which has been fixed, sectioned, and stained in the usual way. The only way to prove that a given cell has latent phagocytic power is to remove it from the body, alive and

uninjured, and under the microscope to watch its behavior. This is what a tissue culture enables us to do in one way. This is what Sabin's supra-vital film enables us to do with even greater ease. In this way it has been possible for us to identify the phagocytic components of the meningeal reactions more completely than would be possible by a study of fixed cells alone, in which only cells with a phagocytic past could be identified and not those with a phagocytic future.

b. Characteristics of phagocytes in supra-vital films

In 1906 Maximow (24) called attention to the fact that his "resting-wandering" cell (our "clasmatocyte" or "wandering endothelial phagocyte") showed a marked affinity for neutral red. He was dealing with a degree of vital staining which we now know to be excessive, since the nucleus was involved; but this observation initiated the wide-spread use of the dye to pick out the phagocytic cells of an exudate. And it is their special reaction to vital dyes in the supra-vital film which enables us to recognize the young inactive phagocyte. The most important differentiation, of course, is from the other mononuclear group, the lymphocytes.

Neither shape nor size, either of nucleus or of cytoplasm, can always distinguish these cells. In size the macrophage may vary from that of an intermediate lymphocyte to several times the size of the large lymphocyte, the size varying roughly with the amount of phagocytic activity which the cell is showing. And the lymphocyte nucleus may be just as kidney-shaped as that of the traditional monocyte, while the nucleus of the monocyte may be quite oval. Both cells may be motile; and shapes vary with motility.

In blood films the character of this motility may be of some help, because the intermediate lymphocyte moves with its nucleus pushing ahead of the cell body, whereas the phagocyte puts out sharp cytoplasmic processes and streams around in a much more vigorous and disorderly manner. In the fluid, however, where all motility is much diminished, this gives us no help.

One learns, therefore, to depend upon the staining reaction with janus-green and neutral-red: (1) In contrast to the large clumped, blue-green mitochondria of the lymphocyte, the phagocytes have few mitochondria, and these are fine and scattered. In the monocyte the inconspicuous mitochondria come to lie at the very outer edges of the cell. In the clasmatocyte the mitochondria may be entirely hidden by the abundant vacuoles except in the smaller, younger cells. (2) Whereas the lymphocyte imbibes little or no neutral-red, the cytoplasm of the phagocyte always contains neutral red, and unless it be a very young phagocyte, the vacuoles will be large and numerous. It is not possible to get the preparation under the microscope and in focus fast enough to catch the cell before it has ingested at least some of the dye. An active mature cell may have its cytoplasm filled with vacuoles, varying in size from fine drops to the size of a red cell. The immature clasmatocyte will always contain at least one or two very large vacuoles, centrally placed, next to the nucleus. The young monocyte begins to take up the dye by staining a fine

ring of granules around a clear space, presumably the centrosphere, near the dent in the nucleus; and it then adds larger and larger vacuoles around this. This fine ring of red granules around a central clear spot is often the crucial differentiating point between a young monocyte and a lymphocyte.

In Figs. 3, 4 and 6 are shown some cells from a stained drop of fluid, which demonstrate the impossibility of telling in fixed smears whether these cells are small monocytes or intermediate lymphocytes. And in Figs. 15 to 27, and 34 to 39 are shown the clear differences between the two groups of cells in supra-vital films.

c. The relationship of the macrophages of the blood stream to those of the connective tissues

The clasmatocyte, the macrophage of the connective tissues, was recognized before it was realized that the monocyte of the blood stream also carried phagocytic potentialities. With this realization, however, the problem arose whether these cells are identical in origin and function, or distinct. The problem is not yet solved, but it is impossible to present clearly our findings in meningeal irritations, without making clear our working hypothesis in regard to the problem in general.

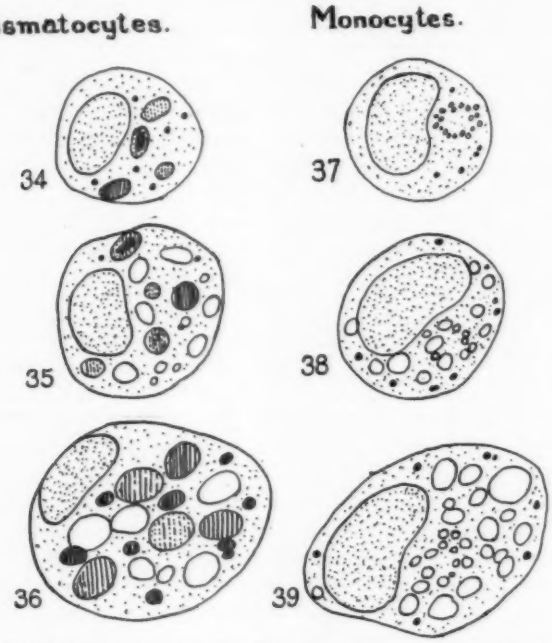
There have been two main lines of inquiry. One group of workers have brought evidence that phagocytes are formed by the transformation of other mature cells. Much of this evidence is very indirect; but Lewis (20) and Lewis and Lewis (21) have actually observed the development of inactive monocytes into highly phagocytic cells in cultures of drops of blood. These engorged cells they believe to be indistinguishable from clasmatocytes.

Sabin, Cunningham and Doan (32, 33), in their recent studies have followed the other line of inquiry. They have attempted to analyze more closely the morphology of the phagocytic cells in supra-vital films. They have then attempted to analyze in detail the reactions of the cells to various types of irritation. And they have attempted to trace the cells back to their origins. Their conclusions are that two distinct morphological entities can be recognized, that these two entities take part differently in inflammatory reactions in the body, and that they are traceable back to quite distinct sources (Cunningham, Sabin and Doan, 9 and 10). One of these entities is the monocyte, the other, the clasmatocyte.

There is no necessary discrepancy between these two studies. Even if we accept the conclusion that there are these two cell-strains, independent in function and origin, it remains possible that under appropriate conditions the one can be transformed into the other. If this can occur in the body, it would mean, for instance, that not all of the clasmatocytes called upon in some inflammatory response had arisen from the clasmatocytic stem, but that some at least had arisen by the transformation of the cells of a collateral branch into cells which were indistinguishable functionally as well as morphologically from the clasmatocyte proper. We cannot yet conclude with safety that the monocytes, which in the studies of the Lewises have developed such great phagocytic activity as closely to resemble clasmatocytes, could in the body function as

clasmatocytes. We do not know enough about the immunological and phagocytic significance of the two cells. But such a functional transformation is certainly within the bounds of possibility.

counts made with the supra-vital film, on the morphological points described by Cunningham *et al.* (9), Sabin *et al.* (32 and 33). These differentiating points are tabulated below, and are illustrated in Figures 34 to 39.



FIGS. 34 TO 39. Sketches of the three functional stages of mature phagocytes in supra-vital films of cerbrospinal fluid. The mitochondria are indicated by solid dots, the neutral red vacuoles by circles of different sizes. The uniform color of these vacuoles in the monocyte series is indicated by the lack of shading,—the varying color of the vacuoles in the clasmatocytes by the shading. The color differences of the neutral red are due, of course, to pH differences.

The characteristic pattern of the monocytes, *i.e.*, a clear zone opposite the "Hof" of the nucleus and the peripheral position of the larger vacuoles and of the mitochondria, are discernible, unless the cell is actively moving or in the most extreme stages of engorgement.

Our study of the phagocytes of the cerebrospinal fluid and the meninges has not added any conclusive data to this problem. The monocytes and clasmatocytes have been recorded separately, however, in all differential

d. The relationship of lymphocyte and phagocyte

Both the observations of the Lewises and those of Sabin and her collaborators argue strongly against the belief

that the lymphocyte is capable of polyblastic transformation into macrophage in inflammatory reactions. Thus, under the conditions of their experiments, the Lewises (20, 21) report no such transformations. And although it may be argued that this transformation can occur only within the body, the fact that their experimental conditions were at once sufficiently normal and sufficiently stimulating to awaken the monocytes to extreme phagocytic activity lessens materially the force of such an objection. And furthermore, the genetic studies of Cunningham, Sabin, and Doan (10) show that the three strains of white blood cells (lymphocyte, granulocyte, and monocyte) can be traced back separately through three distinct

TABLE III

Differentiating points in clasmatocytes and monocytes

GROUP	STRAIN A. "CLASMATOCYTES"	STRAIN B. "MONOCYTES"
(1) Small cells,—believed to be "young" forms, with beginning signs of phagocytic power	Phagocytic activity is shown even in these "youngest" forms by the presence of dye-colored vacuoles and of cellular debris. Mitochondria are abundant in these small forms only	In the "young" form phagocytic activity is evidenced only by the staining of a fine rosette of granules around a clear centrosphere close to the indentation of the nucleus. No large vacuoles and no cellular debris seen. Mitochondria are found in not only the young form but in all the larger forms as well
(2) Medium-sized cells believed to be "adult," —with increasing phagocytic activity	The phagocytic inclusions of cellular debris and of dye-colored vacuoles are scattered throughout the cytoplasm without any pattern beyond a tendency to place the earliest and largest inclusions in the center of the cell next to the eccentric nucleus	Except when the cell is in active motion, or in the very final stage of phagocytic engorging, a definite pattern can be recognized in the ingested material. These granules tend to arrange themselves around the clear centrosphere opposite the "Hof" of the nucleus,—with the larger masses and vacuoles peripherally placed,—and outside of everything else is the scattering of mitochondria
(3) Large, fully active cells, loaded with ingested material	The dye-colored vacuoles are mottled in every shade, showing a wide range of pH	The dye-colored vacuoles tend to conform to a single characteristically salmon-pink shade, indicating a constant pH

chains of ancestral cells to a common origin in the primitive reticular element. Such direct proof of the separate lines of development of these three cell groups does not prove that transformations of one adult form into the others cannot occur, but it makes one demand equally direct observation of such transformations. The observations must be made immediately on the living cell, as the Lewises have done with the monocyte in a blood culture. No sections, whether they be cut from pathological material or from tissue cultures (Maximow 25, 26), can give evidence direct enough to settle the question.

e. The occurrence of phagocytes in the cerebrospinal fluid

1. In fluid from "normal" cats. It is generally held that the lymphocyte is the only normal cell of the spinal fluid. To our surprise we have found young phagocytes in the fluids of cats which appeared to be healthy (Figs. 23–27). Although the existence of transient central nervous system infections has not been demonstrated in cats as it has in rabbits, the possibility of such infections has not been ruled out. We cannot therefore be too sure that the presence of phagocytes in the fluids of cats which seem normal at the time of the examination has no past disease basis.

In supra-vital films of "normal" fluids too few cells are encountered to allow of any statistical generalizations as to the relative proportions of lymphocytes and phagocytes. Therefore, we can only stress the important facts that: (1) the fluid from cats which seem entirely normal and who have no pleocytosis have always yielded us phagocytes on prolonged search and sometimes phagocytes in greater abundance than lymphocytes; and (2) that the smaller of these phagocytes, unstimulated to activity, cannot be differentiated from lymphocytes in stained smears, in tissue sections, or in the counting chamber,—but are usually recognizable at a glance in supra-vital films.

It remains to be seen whether such cells will be found in normal human spinal fluids, where the past normality of the individual can more often be established beyond reasonable doubt. And should normal human beings yield lymphocytes alone, it will be of interest to study those puzzling cases, which everyone encounters, where a central nervous system disease is suspected but where the counting chamber reveals little or no increase in the number of mononuclear cells. A supra-vital analysis of these cells might then yield additional information.

2. Phagocytes in fluid from cats after vital staining of the meninges. An increase in the phagocytes of the fluid can be seen to occur within twenty-four hours after a subarachnoid injection of trypan blue, even during the height of the initial polynuclear reaction. It is not, however, until the polynuclear outpouring subsides that the phagocyte begins to predominate. At this stage, the proportion of lymphocytes present will depend upon what fraction of the fluid is counted (as discussed more fully in the section on lymphocytes). The last drops that are obtainable by spontaneous drainage show a marked rise in the proportion of lymphocytes, so marked in fact that a fluid which has been showing a preponderance of phagocytic cells can

in these last drops show an even greater preponderance of lymphocytic forms.

In the discussion of lymphocytes we have presented our evidence for believing that the explanation of this is an outpouring of the lymphocytic cells from the perivascular channels into the surface meshes of the subarachnoid space, as the pressure within the subarachnoid spaces drops to zero during the drainage. If this be so—and it seems from our sections to be an inescapable conclusion—we are faced with the problem of why phagocytes accumulate in the superficial meshes of the subarachnoid spaces, while lymphocytes accumulate in those tubular prolongations of the subarachnoid space which surround the blood vessels as they penetrate into the brain and cord.

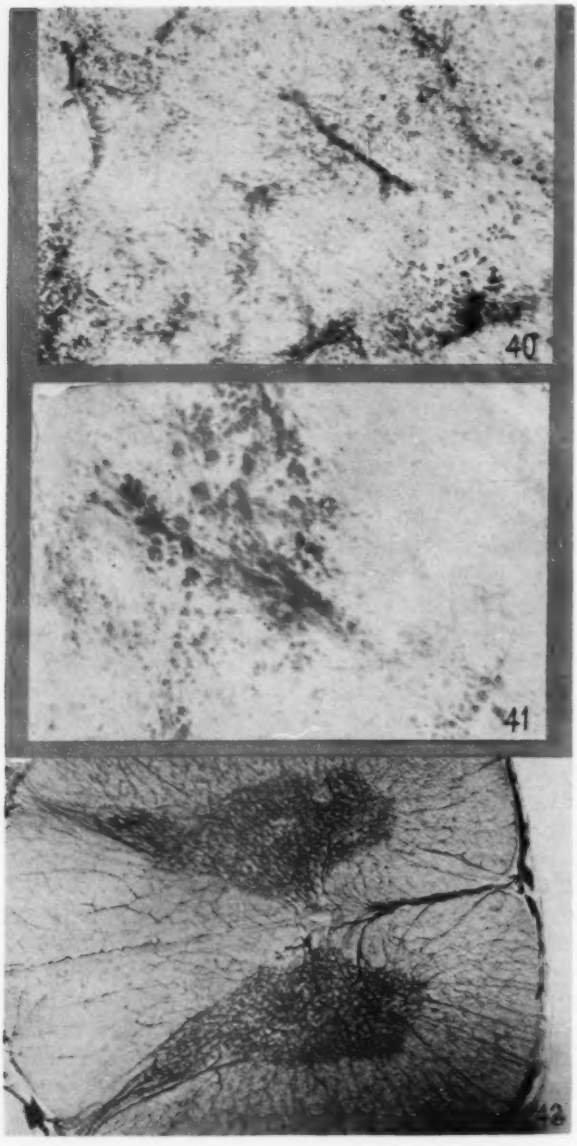
Those who believe in the lymphocytic origin of phagocytes could argue that the lymphocytes in the narrow perivascular channels have not felt the full stimulation of the trypan blue, but that they become transformed into phagocytes on emerging into the open meshes of the subarachnoid space where the concentration of dye is undoubtedly higher. We have direct evidence neither to support nor to deny this interpretation. One would expect, however, that if this were true, transitional forms would be abundant and easily recognized in the supravital film. It is striking, therefore, that no such transitional forms are seen! In stained specimens, as we have pointed out, small phagocytes and intermediate or large lymphocytes may look quite identical and thus simulate transitional stages between the two strains. The living cells, however, separate sharply into their respective groups.

Another explanation is at least as possible as this. It may be that lymphocytes appear equally in the perivascular channels and around the vessels which lie in and on the pia; but that they tend to remain caught in the perivascular channels, while they are washed away from their more exposed position around the pial vessels. The phagocyte, conversely, is more abundant in the superficial meshes of the pia-arachnoid than in its perivascular prolongations; and the explanations of this may be not only that it multiplies more abundantly where the stimulus is greatest, but also that its motility enables it to wander out of the perivascular channels.

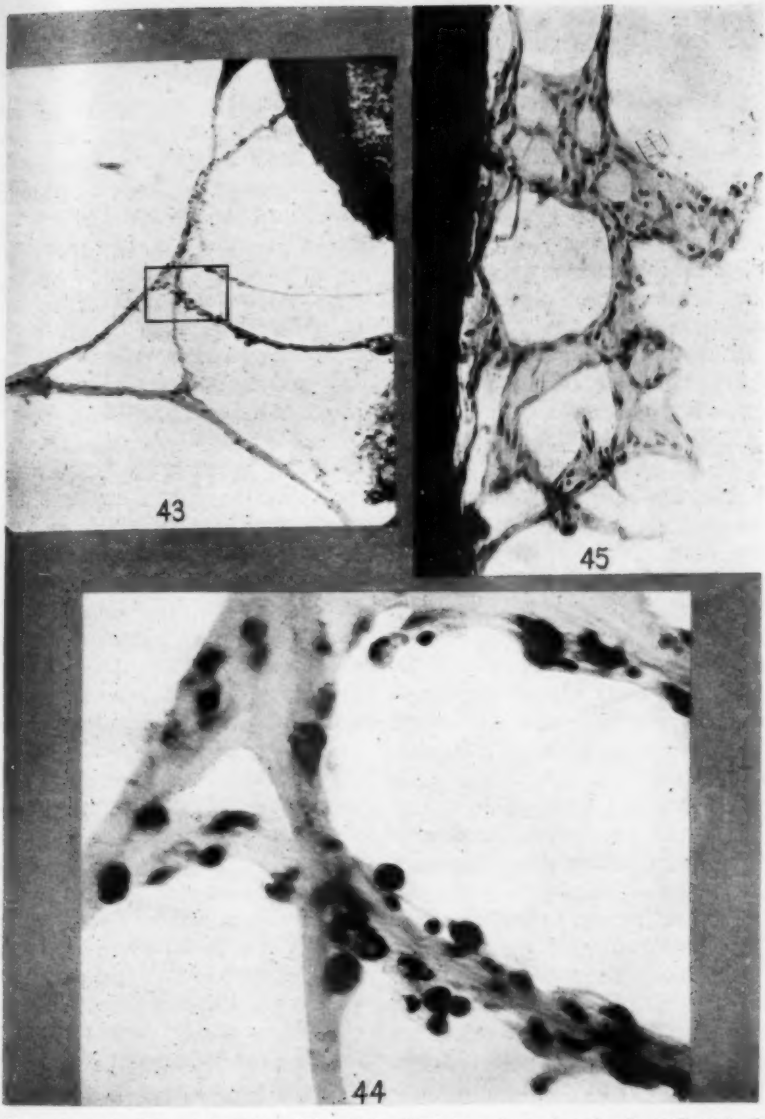
Very few of the phagocytic cells which float freely in the fluid retain the trypan blue. Those which are still clinging to the surface of the meninges are loaded with the blue dye, but when they are free in the fluid, the dye seems to dissolve out rapidly, to be replaced in the supra-vital film with large vacuoles of neutral red. Cells are sometimes met with, however, which contain both dyes.

f. The origin of the phagocytes of the spinal fluid

In seeking the origin of the phagocytes of the fluid it was at first attempted to confirm the position taken by Essick (12). Essick had peeled off strips of arachnoid from formalin-fixed animals after meningeal irritation produced by injections of laked blood into the subarachnoid space. He felt that he could trace every step of a transformation of the flat arachnoidal lining cells into rounded phagocytes bud-



FIGS. 40 TO 42. Figs. 40 and 41 (× 100 and × 200 respectively) showing clusters of macrophages on the arachnoid of Cat 20, after repeated injections of trypan blue. The abundance of double nuclei suggests that these are centers of amitotic multplication. In the section (carmine-stained), most of the macrophages are loaded with trypan blue. The pale cytoplasm of the lining cells, with their faint stippling with fine blue dots, does not show. Fig. 42 shows the amazing network of small vessels in the grey matter of the cord of a normal cat which was injected with india ink. (Preparation loaned by Dr. George Wislocki.)



FIGS. 43 TO 45. Fig. 43 (×70) and Fig. 44 (× 500) show the trabeculae from Cat 20, after severe irritation with trypan blue. The strands are swarming with macrophages which are loaded with blue and highly vacuolated. At places the phagocytes are heaped in layers. The arachnoid lining cells lie underneath and between them, relatively unmodified but for the fine stippling with small dots of trypan blue.

Fig. 45: (×140.) A small blood vessel is indicated by the black band at one edge of the picture. This was peeled out of the pia from the dorsal surface of the cord of Cat 10 (killed during the acute reaction to a second injection of trypan blue). The sheath of pia-arachnoid lining cells can be seen lying close to the vessel, and, arising from this, an elaborate meshwork of trabeculae. It is in this sheath that young clasmatocytes can be found in normal animals. And it is here that polymorphonuclear neutrophilic leucocytes are poured out of the blood vessels into the subarachnoid space during acute inflammatory processes.

ding off and becoming the free cells of the fluid. In our studies of the phagocytes which swarmed over the surface of the spreads of arachnoid and trabeculae, we, on the other hand, felt that the gap between the flat arachnoidal cell and the blue-laden phagocyte remained unbridged by convincing transitional forms.

In Figs. 40 and 41 are shown clusters of phagocytes on a sheet of inflamed arachnoid, and others swarming along the trabecular strands (Figs. 43, 44). But the most minute study of these fixed preparations failed to give us any evidence as to the source of these cells. Even the heavily stimulated arachnoid lining cell seemed to show little tendency to round up. The blue which it ingested was in fine pale particles, and never in the characteristic large vacuoles of the phagocyte. If anything, the lining cell seemed to approach nearer to the serosal cell or fibroblast, under stimulation, than to the phagocyte.[3]

We attempted, therefore, to observe the reactions of the living meninges to the supra-vital film, securing our material both from normal cats and after vital staining of the meninges. Under urethane anaesthesia the cord was exposed and the dura opened with great care. The arachnoid could then be picked up with a very fine hook. By drawing it taut the fine blood vessels in the operative field were emptied, and the delicate membrane was then incised. It was found to be impossible to free an entire thickness of the membrane, for as soon as all four edges were freed, it would curl into a thick little mass of unanalyzable tissue. However, gentle scraping of the under surface of the membrane yielded delicate sheets of arachnoid lining cells, often with the trabecular strands as well; and these could be readily floated onto the filmed slide with a drop of the cat's own spinal fluid.

It was evident at once that such sheets of living arachnoidal tissue contained two types of cells: the true arachnoid lining cell, and the phagocyte. In the normal arachnoid the phagocytes were all immature monocytes, recognizable among the far more numerous lining cells by their smaller size, their more definite outlines, the eccentric oval or kidney-shaped nucleus, and above all by the characteristic ring of fine neutral-red granules around the centrosphere opposite the indentation of the nucleus. The lining cells from these apparently healthy cats contained no dye at all, and showed none of the features of either phagocytic strain nor of their genetic predecessors as described by Cunningham, Sabin and Doan (10) (see Figs. 48–49). On the inflamed membranes, great clusters of engorged phagocytes were seen, loaded with trypan blue (Fig. 46), actively ingesting the neutral red into or around the blue-filled vacuoles, often bi-nucleated as though these clusters were centers of proliferation. In the background were the lining cells, elongated in form, sparsely stippled with a few fine droplets of trypan blue, and taking up equally little neutral red (Fig. 47). Rounded forms of the lining cells were seen as frequently in scrapings from normal cats as from those whose meninges

[3] This leads again into a very controversial field to which we can add nothing conclusive. For a full discussion of the relation of serosal cell to fibroblast see Cunningham (5, 6, 7 and 8).

had been inflamed. This occasional rounding-up cannot therefore be looked upon as a response to irritation (Fig. 49).

We became convinced, therefore, that the monocytes encountered in normal cerebrospinal fluids and in the inflammatory exudates must have arisen primarily by the multiplication of these inactive monocytic forms which seemed normally to inhabit the arachnoid.

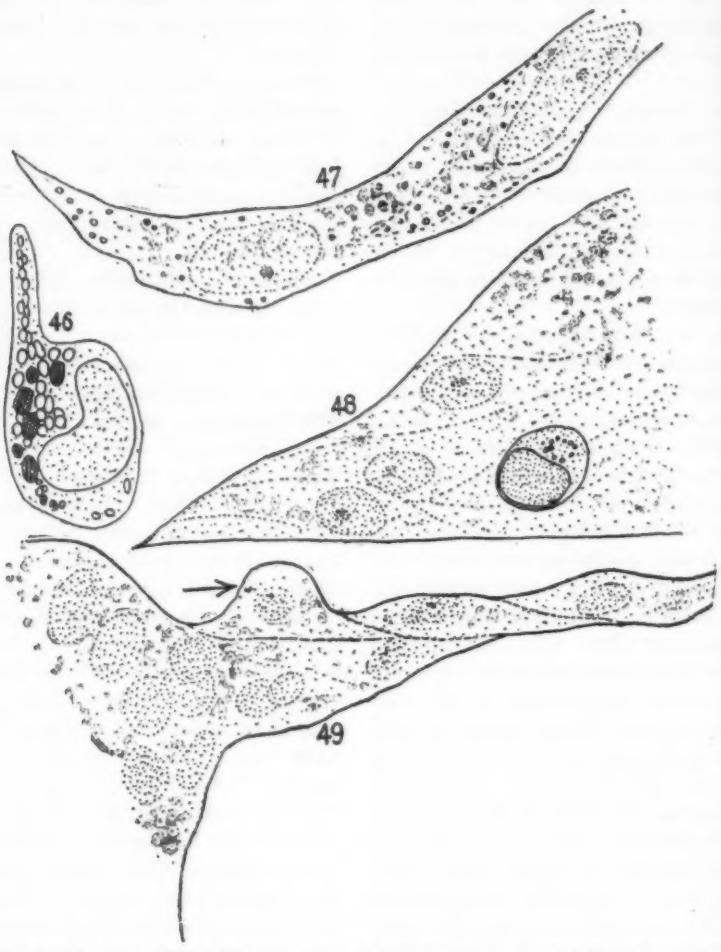
To ascertain the origin of the clasmatocytes, we then attempted to investigate the sheaths of the fine pial blood vessels (Fig. 45). It was found possible to catch the point of a fine hook under one of these minute vessels, and by lifting it gently to force out almost all of the blood. Holding the vessel taut, its pial sheath made a tent, the edges of which could be snipped. The whole thing was then mounted in a supra-vital film. Clasmatocytes, in great abundance, and a scattering of monocytes were present in these vascular sheaths. We do not understand why monocytes alone were found in the normal arachnoid, while both monocytes and clasmatocytes abound in the sheaths of pial vessels from the same animal. It seems clear, however, that it is from the multiplication of these immature phagocytic forms that the phagocytes of inflammatory meningeal reactions arise.

We have been able to find no signs that the endothelium of the vascular bed of the region gives rise to any of these elements. Nor is this surprising when one remembers that none of the vessels that traverse the subarachnoid space lie free within it, but that all are sheathed with a complete coat of pia-arachnoidal lining cells (Fig. 45). There is, therefore, no direct path of access for the blue into the vessels.

Similarly, Foot (14) in his studies of experimental tuberculous meningitis in vitally stained animals could not find sufficient alteration of the local capillary endothelium to account for the invasion of "endothelial leucocytes."

Macklin and Macklin (22) studied brain repair in the rat, vitally staining with trypan blue to identify the phagocytes. They dissected some brains in warm Locke's solution, teasing out fine branching vessels. They state that blue-laden macrophages were found in clumps "attached to," and also "lining" these delicate capillaries. This point in their paper is not illustrated and it is not quite clear what is meant. In our experiments we have never seen a blue-laden endothelial cell. The perivascular channels, on the other hand, lined as they are with invaginations of the pia-arachnoid, show two types of cells down into the very depths of the nervous tissue. One of these is the typical arachnoidal cell, never containing more than a fine stippling of pale granules of dye, and the other is the engorged macrophage. It seems to us that in teasing out a fine vascular tree, as was done by Macklin and Macklin, this perivascular sheath must have been drawn out along with the vessel, showing macrophages attached at times to the outer walls of the vessel, and elsewhere lining what must look like a capillary but was probably a bit of the perivascular channel.

Fig. 42 shows the amazing capillary network in the depths of the nervous



FIGS. 46 TO 49. Figs. 46 and 47 are sketches of cells from a supra-vital film of a meningeal scraping from Cat 50 which had been heavily inflamed with trypan blue. (46) is a macrophage, loaded with trypan blue and with neutral red. (47) represents two arachnoidal lining cells, showing a few fine dots of trypan blue, and a scattering of neutral red vacuoles (*i.e.*, the tiny circles).

Figs. 48 and 49 are sketches of cells from a supra-vital film of a meningeal scraping from a normal cat (Cat 49). (48) shows a sheet of arachnoidal lining cells with one typical young monocyte among them. (49) shows another sheet of lining cells, with a trabecular strand, at the base of which is an example of the way the lining cell in normal animals can round-up (indicated by the arrow).

system, injected with India ink. If, as we believe, each of these minute vessels carries with it a microscopic perivascular channel, lined by an invagination of the pia-arachnoid, and anatomically continuous with the subarachnoid space, it is easy to see how the blue can penetrate to the depths of the tissue, and how a single thin section will often show an apparently isolated blue-laden macrophage far below the surface.

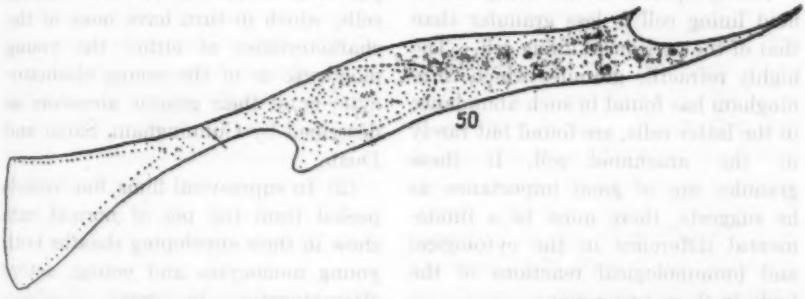


FIG. 50. A single arachnoidal lining cell in a scraping from a normal arachnoid (Cat 49). This cell was at the tip of a trabecular strand and therefore could be watched very closely. It took up no neutral red or janus-green. The filmy cytoplasm had a few fine areas of rarefaction in it, and a thin fish-tail film at one end. It had none of the characteristics of any of the macrophages or their predecessors.

VIII. THE ARACHNOID LINING CELL

We have found the arachnoidal lining cell free in the fluid in only one cat, and that a very young one which had been injected three times and in which the irritation had gone rapidly into the chronic picture in which macrophages abounded. As mentioned above, however, it has been possible to study the arachnoid cell more fully by scraping it from the living membrane and washing it with a drop of spinal fluid on to a supravital film. In such preparations its distinguishing characteristics are essentially those of the less active of the peritoneal lining cells as described by Cunningham (5, 6, 7 and 8) and Sabin *et al.* (33, Figs. 20, 21, 22 and 23). It is a pale translucent cell, with a faint nucleus. The cytoplasm is streaked with a few exceedingly fine rods which do not stain with janus-green, and which therefore cannot be looked upon as mitochondria. It takes up no neutral red unless it has been subjected to irritation (Fig. 50).

After repeated irritant injections of trypan blue the lining cell carries a fine stippling of pale blue granules or vacuoles which in supra-vital films take up some neutral red. Against this background of finely stippled arachnoid cells, great clumps of immense, engorged phagocytes stand out. No transition forms between these two cells are seen, however. Nor do these arachnoid cells in the course of a week's tissue-culture acquire further phagocytic power. It is obvious, however, from the work of Lewis (*loc. cit.*) that these tissues cultures must be pushed further and carried out over longer

periods in diverse media before the possibility of such a transformation can be absolutely denied.

We have found rounded forms of the arachnoid cell, as illustrated in Figure 49, in normal cats, especially along the base of the trabeculae. We do not feel, therefore, that it is possible to deduce from the presence of such rounded cells the conclusion that this rounding up occurs in response to irritation.

The cytoplasm of the living arachnoid lining cell is less granular than that of the peritoneal lining cell. The highly refractile granules which Cunningham has found in such abundance in the latter cells, are found but rarely in the arachnoid cell. If these granules are of great importance, as he suggests, there must be a fundamental difference in the cytological and immunological reactions of the body in these two regions.

In fixed spreads of the arachnoid from cats in which the irritation had been pushed to a chronic stage, the cells on the surface of the membrane were almost all elongated, spindle-shaped cells whose ends seemed to taper out into fiber-like processes. We have been unable to decide whether this meant a conversion of the lining cell into a true fibroblast, or a denuding of the surface with a consequent uncovering of subjacent fibroblasts.

There remains a possibility, of course, that Essick with his injections of laked blood produced a higher degree of irritation than we secured with trypan blue. This might cause a slow increase of the phagocytic action of the arachnoid lining cell analogous to the slow development of phagocytic power by the fibroblast which Maximow (23) described in 1901, and which was also described in detail by Evans and Scott in 1920 (13).

While our evidence, then, is not final, the following facts seem to us to argue strongly against any ready transformation of the arachnoidal lining cell into either free or fixed phagocytes:

(1) The supra-vital technique reveals the presence of young monocytes in normal arachnoids, sharply distinguishable from the arachnoid lining cells, which in turn have none of the characteristics of either the young monocyte or of the young clasmatocyte, or of their genetic ancestors as described by Cunningham, Sabin and Doan.

(2) In supra-vital films, fine vessels peeled from the pia of normal cats show in their enveloping sheaths both young monocytes and young, active clasmatocytes in great numbers. Again, the distinctions between these cells and the true pia-arachnoid lining cells among which they lie are clear.

(3) The arachnoid spreads from cats whose meninges have been vitally stained with trypan blue do not in our preparations show transitions between the weakly phagocytic arachnoid lining cell and the highly phagocytic macrophage. The rounding-up of arachnoid cells cannot be looked upon as a transition, because such rounded forms occur in apparently normal animals.

(4) The heaping-up of layers of macrophages along the trabeculae suggests an active migration of these amoeboid forms along the trabecular strands, rather than a development *in situ*.

(5) No increase of phagocytic power on the part of normal or vitally stained arachnoid lining cells can be seen in tissue cultures of one week's duration.

(6) What transformation the arachnoid lining cell seems to undergo with irritation is rather towards the fibroblast than towards the macrophage.

IX. SUMMARY

(1) The free cells of the cerebrospinal fluid of cats were studied in supra-vital films, and in stained films after fixation by a special method with iodine vapour. The characteristic behavior of the different living cells in supra-vital films is described.

(2) The leptomeninges were studied in supra-vital films, in tissue-cultures of one week's duration, and in fixed spreads.

(3) The cerebrospinal fluid of cats that were apparently normal at the time of examination were found to contain young monocytes as well as lymphocytes. The differentiation of many cells of these two groups is possible only in the supra-vital film.

(4) Supra-vital films of the meninges of normal cats revealed the presence of young phagocytic forms scattered among the pia-arachnoidal lining cells. Young monocytes alone were found on the arachnoid. Both monocytes and clasmatocytes were found in the sheaths of fine pial vessels. No transitions between the lining cell and the phagocytic forms were seen.

(5) Meningeal irritation was produced by repeated injections into the cisterna of 1.0 to 3.0 cc. of trypan blue in distilled water in concentrations of 0.1 to 1.5 per cent.

(6) These injections invariably caused a marked pleocytosis, beginning within a few hours after even the weakest injection.

(7) The fluids of cats with meningeal irritation, produced in this way, contained polymorphonuclear neutrophilic leucocytes, lymphocytes, and phagocytes. The polymorphonuclear leucocytes predominated for the first 24–48 hours, and then were usually outnumbered by mononuclear forms.

(8) In cats in which the irritation had been rendered chronic by repeated injections, a marked difference was found between the first few drops of a fluid to appear and the last that could be obtained. Phagocytes and polymorphonuclear leucocytes predominated in the first sample, and lymphocytes in the last. Evidence is presented for the view that the lymphocytes accumulate in the perivascular channels, and are partly released from these channels by drainage of the cerebrospinal fluid, and can be even more completely washed out by a simultaneous intravenous infusion of hypotonic solutions.

(9) The possibility that this procedure may have definite therapeutic value in certain conditions is mentioned.

(10). The identity of the so-called "plasma cell" with the intermediate lymphocyte is suggested and discussed.

(11) Evidence is presented for the independence of the phagocytes of the fluid from either the lymphocyte or the arachnoid lining cell. Their origin from young phagocytic forms existing normally throughout the meninges is proved to be possible, by the demonstration of the presence of such young phagocytes in normal arachnoid scrapings.

(12) The specifically serosal nature of the arachnoid lining cell and its uncertain but possible relationship to fibroblastic activity is discussed on the basis of the reactions of these cells in tissue cultures, in supra-vital films, and in fixed preparations.

(13) The cerebrospinal fluid accelerates the degeneration and inhibits the motility of the polymorphonuclear neutrophilic leucocyte and of the lymphocyte in a marked degree. It is less destructive to the eosinophilic leucocyte and to the phagocyte.

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Argentaffine Tumors of the Appendix and Small Intestine

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Review of the Literature

In 1838 Merling[1] reported a case of primary carcinoma of the appendix. In this report no microscopical evidence was given of its true carcinomatous character, but judging from the general description, the case, it seems, may rightly be considered as such. So far as can be determined from a complete review of the literature on the subject, Merling's case represents the first on record of a new growth in the appendix of a carcinomatous character. Primary neoplasms of the appendix are of such rare occurrence, as are likewise primary growths of the entire small intestine, that they have usually been reported. The total number of cases of appendiceal carcinomata found in the literature up to 1923 was 317 (Jackson (43)). To this number have been added several recent cases. It would seem, therefore, of little importance to place on record further cases, all of which are characteristic and similar in every respect to those already described. And so, were it not for the fact that the true pathological character of the primary tumors of the appendix and small intestine has been of late the subject of such widespread interest, and their clinical and pathological characteristics the subject of such varying interpretation, a report of further cases would hardly seem justifiable. The recent change in the viewpoint of pathologists regarding the origin of the so-called carcinoma of the appendix and small intestine is of considerable interest and importance, and for this reason we have attempted to trace the development of our knowledge of these peculiar tumors, and here present our own cases as illustrative of this neoplasm which for so long has been a pathological puzzle.

Between 1838 and 1875, few cases of primary neoplasm of the small intestine and appendix are found in the literature. Kolaczek's (1) case, described as "adenoma destruens" was apparently a true cylindrical cell carcinoma, in view of the fact that there were generalized metastases. This case is mentioned here simply because it is representative of many so incompletely described that one cannot be sure that the tumor actually arose from the appendix. Many carcinomata are recorded as primary in the appendix or jejunum, which, in truth, should be regarded as secondary to a primary growth in the adjacent

[1] Original paper not accessible. Cited by Elting (11).

segment of the large intestine. In 1882, Berger (2) reported a case of cylindrical cell carcinoma which seems clearly to have arisen from the appendix.

The first contribution to the subject of primary carcinoma of the appendix and small intestine which seems of any actual value was made by Lubarsch (3) in 1888, in a paper in which he described two cases. The first showed at autopsy two small tumors located in the ileum. In the second, six small tumors were found at autopsy in the lower ileum of a man aged 52 years. All of the tumors are described as small nodules of whitish tissue, occupying the submucosa and the muscularis. They were composed of nests and strands of epithelial cells which were regarded as strikingly unlike the ordinary cylindrical cells of the intestinal mucosa and, hence, quite unlike the cylindrical cells which form the ordinary adenocarcinoma found elsewhere in the gastro-intestinal tract. However, by serial sections through the tumors it was demonstrated that the epithelial nests and strands were definitely connected with the crypts of Lieberkühn, and that they most likely represented an outgrowth from the glands of the intestinal mucosa. A pseudo-glandular arrangement of the cells was described, *i.e.*, small spaces were found within the cell nests, giving the general appearance of a glandular lumen about which the tumor cells were arranged radially. The presence of a pink-staining, colloidal, amorphous substance in these clear spaces led Lubarsch to the conclusion that they were not really lumina but that they represented vacuolated spaces resulting from necrosis in the cell masses. Judging, then, from the extraordinary features of the tumors, to wit: (1) multiplicity of tumors, (2) lack of metastasis, (3) absence of true glandular structure, and (4) dissimilarity of the cells from those of the normal intestinal mucosa, it was quite apparent that, although these tumors were unquestionably of epithelial origin and traceable to the crypts of Lieberkühn, they were, however, distinctly different from the tumors of adenocarcinomatous character occurring elsewhere in the gastro-intestinal tract. Although these striking differences were fully appreciated, Lubarsch was content to speak of his cases as "primary carcinomata of the ileum."

Between 1888 and 1900, numerous new cases of primary tumors of the appendix and the small intestine were reported. Ransom (4), in 1890, described a case of a small tumor in the ileum which extended through the peritoneal covering and into the mesentery for a short distance. Microscopically, the new growth was like Lubarsch's tumors. The important point in connection with this case was the appearance of multiple secondary tumors in the liver identical in character with the tumor in the ileum. In spite of the freedom of the mesenteric lymph nodes from metastases, the origin of the liver tumors seemed beyond question to be the small tumor of the ileum, for careful search throughout the body revealed no other neoplasm. The ability of this special type of epithelial tumor to metastasize was later, in 1896, confirmed by Notthaft (5), who described a case in which three small

tumors were found in the jejunum, about 10 cm. from the duodenojejunal junction. The mesentery had been invaded by one of these tumors. In the immediate vicinity nests of tumor cells were found in the lymph glands.

In 1895, Glazebrook (6) described a case in which a tumor, the size of a pigeon's egg, was found in the appendix, without, however, any extension to the mesentery or mesenteric lymph glands. The description of the tumor, though quite inadequate, seems definitely enough like that of the tumors of Lubarsch, Ransom and Notthaft, to warrant its being grouped with them. Glazebrook, however, did not consider the tumor epithelial in origin. Because of its peculiar relationship to what he considered greatly dilated lymph spaces and blood vessels, he applied to it the term "endothelial sarcoma," with, however, quite inadequate proof of the endothelial source of the tumor cells.

Many statistical studies of carcinoma of the appendix were made between 1900 and 1907, but very little can be found in the literature during this period which helps to clear up the problem of the true nature of the little tumors. Numerous cases were reported, with lengthy discussions of the clinical manifestations of the tumor and its diagnosis. However, in no case reported is one justified in stating that the tumor as such could be diagnosed. Some of the cases were discovered at autopsy, having given rise to no symptoms or signs during life. The appendiceal tumors were usually found at operation, in which case they were practically always associated with an acute or chronic inflammatory condition in the organ for which the operation was performed. Many of the tumors were never recognized until the microscopical sections were studied. The neoplasm was considered by Hurdon (9), who reported a case in the *Johns Hopkins Hospital Bulletin* in 1900, as clinically dangerous, especially because it produces no signs or symptoms *per se* which would indicate its presence during any stage of its development.

A. O. J. Kelly (10), writing also in 1900, described four cases, one of which he designated as an endothelioma, possibly following the lead of Glazebrook (6). One of the cases was most probably not primary in the appendix, but a metastasis from a tumor elsewhere in the abdominal cavity. Kelly called attention to the associated obliteration of the lumen of the appendix in one of his cases, a feature which will be seen later to be of some considerable significance in connection with the true nature and origin of these primary growths of the small intestine and the appendix.

In 1903, Elting (11) reviewed all the early literature and analyzed all the cases critically which had been reported up to that date. He found, after ruling out all inadequately described cases and all cases which were possibly primary in other organs, only 23, including 3 of his own, which were unquestionably of appendiceal origin. He did not study the tumors which were present in the small intestine.

Bunting (14), who described one case of true cylindrical cell adenocarcinoma and one of the quite different type under discussion, drew attention to the biological and morphological similarity between the primary tumors of the appendix and small intestine

and the basal cell epitheliomata described by Krompecher (53). In his paper, written in 1904, he describes a case in which six small tumors were found in the ileum, all of which were confined to the intestinal wall and none of which showed any metastases. A direct connection between the crypts of Lieberkühn and the nests of tumor cells in the submucosa was found in serial sections.

The rather uncertain character of the tumor under consideration remained in this confused state until the meeting of the German Pathological Society in Dresden, in 1907, before which Oberndorfer (18) discussing the subject in detail, came out clearly and unreservedly for a separation of this tumor from the group of true carcinomata, and for placing it in a group of its own, for which he proposed the term "carcinoid." His discussion was based upon a study of 6 cases of multiple tumors of the small intestine. His description of the individual tumors is classical and, although obviously incorrect in some particulars, may be summarized as follows: (1) The tumors are usually multiple; (2) the cells are largely undifferentiated masses, but may show glandular form; (3) they are circumscribed and have no tendency to infiltrate the surrounding tissue; (4) they do not metastasize; (5) growth is slow, no large size being attained; (6) the tumors are of a harmless character; (7) they lie regularly in the submucosa, but may send processes into the mucosa; (8) the muscularis mucosae is always present; (9) the stroma is smooth muscle derived from the muscularis mucosae.

These characteristics being essentially of non-malignant variety, the term "carcinoid" instead of carcinoma seemed desirable and fully justified. Regarding the origin of the multiple carcinoids of the small intestine, Oberndorfer would make no definite statement, but preferred to consider them as belonging to a general group of malformations, which Trappe (19) had previously discussed as the most probable source of origin for the adenomyomata of the small intestine.

According to Trappe, the origin of both the carcinoids and the adenomyomata could be found in pancreatic rests which are of fairly common occurrence in the gastro-intestinal tract. He thought that, although the two formations had a common origin, they might well enough be differentiated from each other morphologically. The adenomyoma was regarded as the most highly developed outgrowth of these rests because of their being composed of well differentiated glandular tissue, associated with well formed ducts which were found to communicate with the crypts of Lieberkühn. The carcinoid, on the other hand, represented a very low order of development from the pancreatic rest, in which the epithelial cells had undergone no differentiation but remained in a disorganized state with, however, an unusual capacity for growth.

Such a conception of the nature of the carcinoid appealed to Oberndorfer (18) more than Bunting's view (14) that the tumors were analogous to Krompecher's basal cell tumors of the skin.

Two years later, in 1909, Burkhardt (25) studied a group of carcinoids of the small intestine and differentiated

them from adenomyomata and accessory pancreatic formations by (1) their lack of a muscular stroma, (2) absence of pancreatic acini, (3) absence of ducts, (4) constant connection of the tumor nests with the crypts of Lieberkühn, (5) production of metastases, and (6) invasive character of the growth. Contrary to the conception of Oberndorfer (18) and in support of Bunting (14), Burkhardt (25) considered the carcinoids definitely of basal cell character, and analogous to the basal cell cancers of Krompecher. This conclusion he based upon the following facts: (1) The tumors are derived directly from the intestinal epithelium; (2) they are of low degree of malignancy; (3) they possess a hyaline fibrillar stroma; (4) the cells are undifferentiated.

Toenniessen (28), in 1910, also objected to the conception that the carcinoids are derived from accessory pancreatic tissue and are identical with the adenomyomata; on the contrary, he preferred to think of them as the result of development of submucous rests. He suggested that they might represent glands of internal secretion in view of a hitherto undescribed organoid character, and remarked upon a similarity between the cell nests of carcinoids and the islands of Langerhans. This conception of the tumor was developed further by Saltykow (32, 33) in two separate papers published in 1912–1913. In one of these (32), read before the German Pathological Society, carcinoids were considered as derived from pancreatic rests, just as were the adenomyomata. They were regarded as developments, however, in which only islands of Langerhans were produced, whereas, in the adenomyomata, glandular structures with ducts and a supporting framework of connective tissue developed from the pancreatic rest. In the discussion of the paper, M. B. Schmidt agreed to the pancreatic rest origin, but doubted the pure islet character of the carcinoid. At the same time Oberndorfer called attention to the fact that the protoplasm of the carcinoid cells gives a striking chrome reaction, and contains an abundance of doubly refractile substances. In his second paper (33), Saltykow restated his pancreatic rest hypothesis and sought to establish its truth on morphological and embryological grounds. It is here that one finds the fullest and most valid evidence for the pancreatic rest origin of the carcinoids, though this was to be shown in error later by Gosset and Masson.

In 1910 it had been suggested by Hübschmann (50) that the carcinoids, because of their yellow color as seen in the gross, had their origin probably in the "gelben Zellen" of the intestinal mucosa, the chromaffin character of which had been established by J. E. Schmidt (49) in 1905. Schmidt thought that these "gelben Zellen" were most likely the same as a peculiar type of cell of the mucosa described by Kultschitzky (48) in 1897, although he was unable to prove the identity of the two.

Gosset and Masson (38), in 1914, restudied the chromaffin cells of the intestinal tract and after applying silver impregnation method described them as follows: (1) they occur in the depth of the crypts of Lieberkühn throughout the gastro-intestinal tract;

(2) the cells are situated between the cylindrical cells of the mucosa, some extending from basement membrane to lumen while others remain without contact with the lumen; (3) "argentaffine" or silver-staining granules are always demonstrable in these cells and are always located between the basement membrane and the nucleus; (4) there are vacuoles in the cytoplasm which are probably the result of elaboration of the granules; (5) these cells form an endocrine gland of endodermic origin similar to the islands of Langerhans; (6) the argentaffine cells may proliferate and form a conglomerate (organoid) tissue.

Hübschmann did not apply the chrome reaction to his tumors and therefore did not know of the characteristic behavior of the tumor cells to the chrome compounds. It was not until 1912 that the characteristic chrome reaction was noted, and it was then that Oberndorfer described it in the course of his discussion of Saltykow's paper, as stated above.

Gosset and Masson (38) studied the carcinoids by methods similar to those which had been applied to the intestinal mucosa and came to the conclusion that the cells which form the tumor closely resemble, and are probably identical with, the cells of the chromaffin tissue of the paraganglia which are filled with argentaffine granules. The fact that the tumor cells contain large quantities of lipoids was found to be the one important point of differentiation between these two otherwise similar types of tissue. A microchemical similarity was also found between the carcinoid cells and the cells of the medulla of the adrenal. Morphologically, the similarity of the tumor cells to the cells of the islands of Langerhans had been previously pointed out by Saltykow. All of these facts seemed to point to some common relationship between the well known chromaffin tissue and the tumor under discussion. In view of the microchemical and morphological identity of the chromaffin cells of the crypts of Lieberkühn and those of the carcinoid tumors, the latter were regarded by Gosset and Masson as tumors of endocrine origin and hence as "endocrine tumors."

This conception of the carcinoids was not by any means universally accepted. Krompecher (42), in 1919, undertook a study of these tumors in connection with certain other neoplasms of a somewhat similar character, as a supplement to his earlier work on basal cell cancer, and, without mentioning Masson and Gosset in any way, came to the conclusion, based entirely on morphological data, that the carcinoids are basal cell tumors analogous to the basal cell cancer of the skin, and that they might occur in any one of three forms, (1) solid, (2) adenoid, and (3) cylindromatous. There seemed to him two possibilities of origin, (1) from the basal cells of the crypts of Lieberkühn, and (2) from displaced cylindrical epithelium in the form of epithelial rests.

The findings of Gosset and Masson were confirmed by Hasegawa (44), in 1923, and by Danisch (51) in 1924. In view of Engel's (45) report that embryonal rests of the submucosa may be responsible for the origin of carcinoids, Hasegawa concluded that the carcinoids might arise either directly from the epithelium of the crypts of Lieberkühn, the cells of Kultchitzky

and J. E. Schmidt, or from the epithelial rests described by Engel.

Masson (46) reviewed the whole subject of carcinoid tumors of the gastro-intestinal tract in a paper published in January, 1924, and reported a detailed study of the argentaffin cells of the intestinal mucosa. No new facts, however, were brought out in connection with the origin of the tumors in question.

The most recent contribution on the subject of the carcinoids is by McCordock (52). The work of Masson had not come to his attention at the time of his publication and so he was inclined to favor the tissue malformation origin of the tumors.

In *summary*, then, one finds in the literature a multiplicity of opinions regarding the nature and the origin of the small carcinoid tumors which occur throughout the gastro-intestinal tract. The more important of these may be listed as follows:

1. The tumors represent true carcinomata derived from the epithelium of the gastro-intestinal mucosa.

2. They may be included in the group of "basalomata" analogous to the basal cell cancers of the skin.

3. They may be malformations belonging to the general group of tumors developing from the pancreatic rests, as adenomyoma, and accessory pancreas.

4. They may be tumors derived from the chromaffin cells of the crypts of Lieberkühn and hence be endocrine tumors or tumors of the paraganglionic system.

It would seem that, all things being considered, the weight of evidence is in favor of the endocrine origin of the tumor in question and that they may be rightly called "endocrine tumors," or probably better "argentaffine tumors," and permanently separated from the general group of carcinoma.

So far as we have been able to determine in going through the literature, the argentaffine character of the tumors under question has not come to the attention of American and English writers on the subject, and so it seemed desirable to present this short review in connection with the following cases, which are reported as typical examples of the "argentaffine" tumors.

CASE REPORTS

Case 1. Autopsy 6032. Figure 1.

Clinical history. The patient was an old woman, aged 64, who was brought into the hospital in coma following a fall while on the street car. The coma persisted, associated with signs pointing towards a cerebral lesion on the right side. Blood was found in the spinal fluid. The clinical diagnosis was cerebral haemorrhage. The patient was in the hospital for seven days. Little change was noted in the condition throughout this time. At death the clinical diagnosis was chronic nephritis, hypertension, arteriosclerosis and cerebral haemorrhage.

Anatomical diagnosis. Arteriosclerosis; cerebral apoplexy; widespread senile atrophy of all the organs; duodenal ulcers; chronic tuberculosis of the lungs with cavity formation, terminal lobular pneumonia; fracture of ribs; bruise on the right arm; a spinal curvature; emphysema of the lungs; "carcinoma" of the stump of the appendix.

Macroscopical note. The tumor is a small nodule, about 5 mm. in diameter, which occupies the stump of the appendix. Its exact relationship to the mucosa cannot be made out from the section. It is, however, completely surrounded by a circular band of muscle which apparently represents the inner coat of the muscularis. Muscle fibers from this coat can be seen at one part extending directly into the tumor mass.

The tumor tissue is composed of strands

and small masses of round or oval cells embedded in an eosin-staining, hyaline stroma. Almost all of the nests and strands of cells lie within spaces in the supporting tissue. Small blood vessels of capillary size are seen in the supporting tissue of the tumor but none of them contain tumor cells. The more one studies these spaces filled with the tumor cells, the more one is impressed with their lymphatic-like appearance. In places flattened spindle cells exactly like endothelium are seen lining the spaces, although no place can be found where the spindle cells form a complete sheath about the tumor mass. No blood cells are found in contact with the tumor cells. Sometimes the cells distribute themselves about the space in such a way as to form an alveolar-like structure with a rather atypical and irregular lumen. This appearance seems, however, to be only an artefact. A true gland-like arrangement is quite definitely not present. In the more solid nests of cells no alveolar arrangement whatever is seen. The cells of the tumor are not confined entirely to the nodular mass in the appendix; a few strands and cell nests can be seen in the circular layer of muscle. The tumor is, therefore, not encapsulated, but has very definite invasive and infiltrative characteristics.

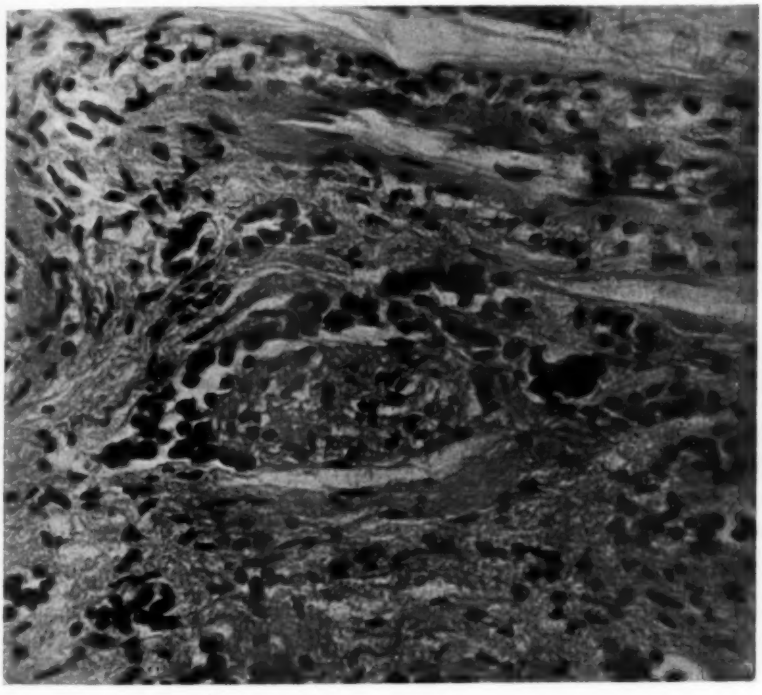

FIG. 1. CASE 1 (AUTOPSY 6032). ARGENTAFFINE TUMOR OF THE APPENDIX

In the center of the photograph a large nerve bundle is surrounded by tumor cells. Nests of the tumor cells are also seen embedded in a dense connective-tissue stroma. There is no regular arrangement of the tumor cells.

The cells which compose the tumor are round and oval in shape. The nuclei are round, comparatively large, quite regular

in shape, uniform in size, and rich in chromatin, which is distributed diffusely, though coarse granules may be found in almost all of the nuclei. Nucleoli are quite frequently seen. The cytoplasm is abundant in some cells, but in the greater part of the tumor it is only moderate in amount in comparison with the size of the nucleus. Coarse granules which have a rather reddish-brown color are found in most of the cells. Cytoplasm that contains no granules, on the other hand, is full of vacuoles. Nowhere can cells be found which have a clear-cut columnar form, like the cells of the crypts of Lieberkühn.

The stroma which forms the greater part of the tumor nodule consists largely of hyaline connective tissue. Small blood vessels are fairly common, but there are a few larger ones. Nerve fibers are numerous and present an interesting relationship to the tumor cells. In places the cells form an interrupted sheath about the nerve fibers. Toward the periphery of the nodule can be seen small groups of smooth muscle cells which are continuous with the circular coat of muscle surrounding the tumor. No muscle can be found with certainty within the tumor mass, although there are numerous isolated groups of spindle cells which morphologically are like smooth muscle. These do not, however, react characteristically to the differential stains. Nowhere can there be found any supporting stroma within the cell nests and strands. The cells are simply packed loosely together without any intervening tissue. Isolated groups of tumor cells, and sometimes single cells, can be seen far out in the muscle coat and in what appears to be the serosa. No metastases are found in any of the internal organs.

Case 2. Autopsy 6739. Figure 2.

Clinical history. One year before death the patient complained of fullness and a sense of constriction about the upper abdomen. Gastric obstructive symptoms appeared and became gradually more pronounced. The patient lost weight progressively during the five months preceding operation. Laparotomy demonstrated an inoperable carcinoma of the stomach. Death followed the operation.

Anatomical diagnosis. Carcinoma of pylorus and lesser curvature of the stomach. Metastases to liver, diaphragm, lymph glands; "basal cell epithelioma of jejunum"; hydronephrosis; pyelonephritis; lobular pneumonia; tuberculosis.

Microscopical study: The tumor is a small nodule, 5 mm. in diameter, located in the wall of the jejunum. It has a round or oval shape and projects into the lumen of the jejunum rather than producing a nodular projection on the peritoneal surface of the intestine. The tumor nodule lies in the mucosa and the submucosa. The muscularis mucosae can be quite definitely traced over the whole tumor; it becomes much thinned out and the muscle cells quite irregularly arranged. Some muscle strands are seen to dip down for a short distance from the muscularis mucosae into the trabeculae of fibrous tissue which form the stroma of the tumor. The mucosa appears infiltrated with tumor cells, although the muscularis mucosae, as described above, is fairly well preserved in the section studied. The mucosa is compressed by pressure from below due to the presence of the tumor. The epithelial covering is absent, only compressed and atrophic remains of the villi being present. There are places where the tumor-cell nests with their surrounding stroma are seen to come directly in contact with the lumen of the intestine. No tumor can be seen in the muscular layers, in either the inner or the outer coats, although the tumor cells are present in the compressed submucosal connective tissue, which lies on the inner coat of muscle. There is no definite capsule, though impression of one is given by the compressed submucosal connective tissue which surrounds the tumor. It is quite obvious that this tumor is distinctly different from the tumor of the stomach, which on microscopical examination is seen to be a characteristic cylindrical cell adenocarcinoma.

The cells which form the tumor are of the characteristic argentaffine type. They are round, oval and occasionally low cylindrical, depending upon their location and their relation to the supporting stroma. Those in the central part of cell nests are round; toward the periphery they become oval, and at the edge of the cell nest they are low

cylindrical in type with the long axis perpendicular to the surrounding connective tissue stroma. When the cells are under extraordinary pressure as a result of being packed in small spaces, the most peripheral ones are flattened in such a way as to form spindle-shaped cells with the long axis running in the direction of the circumference of the cell nest. Under such conditions the cell mass seems to be bordered by a layer of cells similar to the germinal basement layer of cells seen in glands and ducts of the breast where the epithelium is undergoing unusual hyperplasia. The nuclei are round, oval or spindle, corresponding to the shape of the cell. They always stain rather deeply, having an abundance of chromatin material, but some of the smaller nuclei are more deeply stained than others. These suggest chromatin

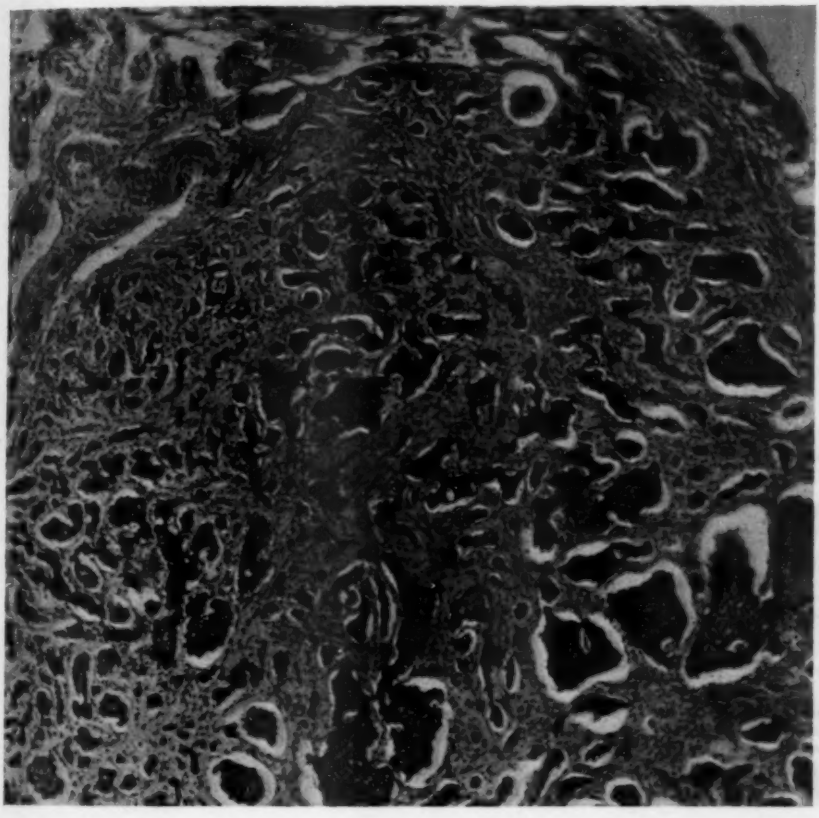

FIG. 2. CASE 2 (AUTOPSY 6739). ARGENTAFFINE TUMOR OF THE JEJUNUM

At the right upper corner of the photograph a small bit of the jejunal mucosa is seen. The muscularis mucosae is well preserved. Strands of smooth muscle extend into the tumor from the muscularis mucosae. Nests and strands of argentaffine cells embedded in a rather dense fibrous stroma form the neoplasm. No glandular arrangement of the cells is present.

changes in early mitosis, but nowhere are karyokinetic figures seen. In some nuclei the chromatin material is arranged in coarse granular clumps, giving the nucleus a vesicular appearance. The cytoplasm is granular and of moderate quantity. In some cells vacuolization is especially well marked.

As already mentioned, the cells are arranged in nests and strands which anastomose quite freely. These cell groups lie in spaces formed by the supporting stroma which they usually fill completely. Some fields show incompletely filled spaces, but it seems quite obvious that the condition is due to shrinkage and is therefore an artefact. So far as can be made out there is no supporting stroma within the cell mass. Occasionally, however, one may find small blood vessels of capillary or arteriole size embedded in the cell groups. The cells sometimes are arranged radially about such a structure, giving the impression of a gland-like formation with a central lumen. However, no true glandular formation is observed throughout the extent of the tumor.

The stroma is formed by coarse trabeculae of fibrous tissue, sometimes compact and sometimes loose. Toward the periphery of the tumor can be seen smooth muscle, which is continuous with the muscularis mucosa. No muscle is recognized in the deeper parts of the tumor mass. Nerve fibers may be seen, but they are not numerous. Lymph channels are quite inconspicuous.

Case 3. Surgical Pathology No. 21703. Figure 3.

Clinical history. The patient was a white male adult who had complained of vague gastro-intestinal symptoms for two years. An exploratory laparotomy was performed because of suspected "stasis" in the caecum, which was thought possibly to have resulted from recurrent inflammation of the appendix. The appendix was found curled upon itself like a cockle-shell. The mesentery of the appendix showed some induration. The appendix and indurated mesentery were removed *en bloc*. Small normal lymph glands about the proximal end of the appendix were observed and removed. Inspection of all other abdominal viscera revealed no lesions.

Gross description note. The appendix is curled upon itself in a cockle-shell-like fashion, as mentioned above. At the site of the curve there is a tumor mass which seems circumscribed, but which doubtless occupies the wall of the appendix and the adjacent peritoneal tissue.

Microscopical study. The tumor is seen to occupy a position within and without the wall of the appendix. The appendix is doubled on itself in such a way that the section shows two cross-sections of its walls side by side with some intervening fibrous tissue. The lumen of the appendix is almost completely obliterated. In each cross-section there remains only a small, irregular canal, lined with convolutions of epithelium, the cells of which are almost entirely of the mucous, or goblet, variety. There can be found only small collections of lymphocytes, and no characteristic lymph follicles with germinal centers. The lymph folicles, together with the loose submucosal tissue normally infiltrated by wandering cells, have been replaced by fibrous tissue arising from the submucosa.

The submucosa consists of a mass of rather coarse, fibrous connective tissue, in which nerve fibers are conspicuous. This tissue becomes hyalinized just beneath the basement membrane of the remaining epithelial cells of the mucosa. Embedded in this fibrous tissue, in spaces separated by dense bands of connective tissue, are nests and strands of tumor cells. The line of demarcation between the submucosa and the muscle coats is quite distinct in most places. In some places, however, the tumor is found invading the muscularis as well as the submucosa, and appears here, as in all other cases, in nests and strands of round cells.

There seems to be no definite relationship between the nerves, vessels, lymphatics and the tumor cells. It is quite clear in this case that the tumor has extended beyond the walls of the appendix and into the mesenteric fat. This is borne out by a second section from the case, taken from the mesentery adjacent to the tumor. Here there is extensive invasion of the mesenteric fat by cords and nests of the tumor.

The cells have essentially the same character as those described in the preceding cases. The vacuolated character of some of

the cells and the abundance of the coarse brownish granules in others is more striking than seen in the previous cases. An additional feature in connection with the cell nests is the presence of irregularly shaped spaces filled with a necrotic pinkish material, which shows needle-like, clear areas, obviously the site of crystals which have been dissolved out by the technical procedures (Fig. 4). The presence of these crystals in such areas is a feature which we have not seen in any other cases and which we have not found described in the literature. It is possible that the crystals may represent a substance elaborated by the cells of the tumor. Unfortunately, lack of material in this case makes a further study of this crystalline substance impossible.

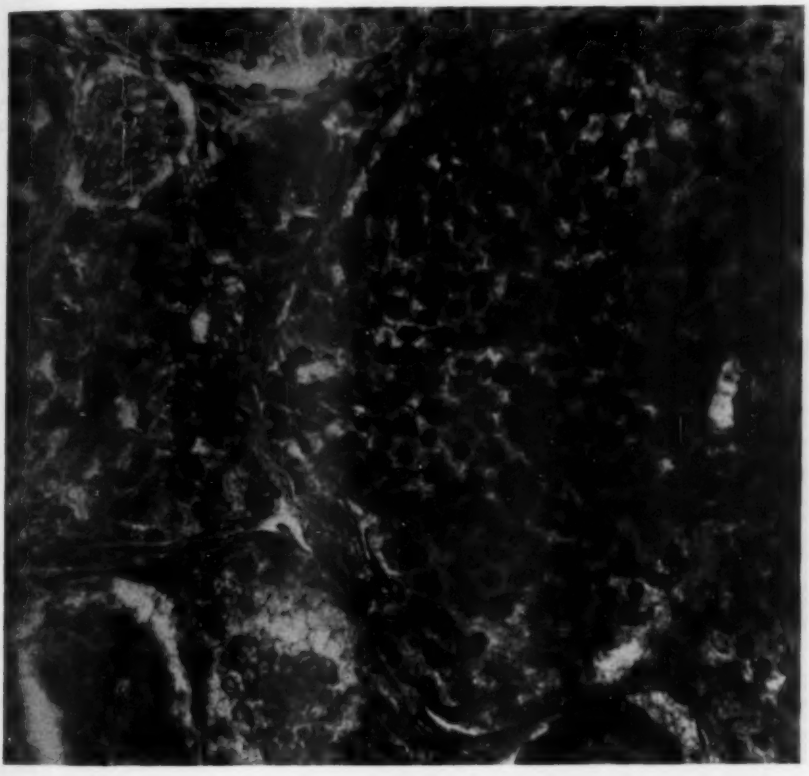

FIG. 3. CASE 3 (SURG. PATH. NO. 21703). ARGENTAFFINE TUMOR OF THE APPENDIX

High-power photograph of nests of argentaffine cells encroaching upon the lumen. Along the lower margin of the figure are three crypts of the mucosa separated from the adjacent tumor cells by a thin connective-tissue membrane.

Case 4. Surgical Pathology No. 34485. Figures 5, 6, 7.

Clinical history. The patient was a young white woman, aged 27, who complained of sharp pain in the right upper part of the abdomen. The original attack of pain dated from four years previously. The symptoms had recurred some twelve

times after the original attack. The most recent attack had been associated with some nausea and vomiting. No jaundice, or fever had ever occurred. Examination on admission revealed some tenderness over the gall-bladder region. No signs were found pointing to an appendiceal inflammation. Slight jaundice was observed during the pre-operative stay in the hospital. A laparotomy was done and the gall-bladder and appendix removed.

Gross description. The gross specimen consists of an appendix which measures about 5 cm. in length. The organ is quite atrophic, its diameter being only about 4 mm., except at its most distal portion where it gradually expands to form a nodule, which measures 5 to 8 mm. in diameter. Over this nodule one sees numerous dilated blood vessels. The fat of the mesentery is rather densely adherent to the tumor. Showing through the peritoneum and through a thin fatty covering, one sees a pale, grayish tumor, which is umbilicated. This mass at the end of the appendix is very dense and almost stony-hard on palpation. On section through the tumor one sees a coarse mass of grayish fibrous tissue, in which are embedded numerous minute, fairly discrete, areas of a yellowish color. The remainder of the appendix appears to be normal.

The second gross specimen consists of a very much distended gall-bladder, whose walls are considerably thickened. On opening the gall-bladder one finds several large stones and many smaller ones. One of the larger gall-stones occupies the region of the cystic duct, but it apparently does not occlude it, as it is quite easy to remove it from its location.

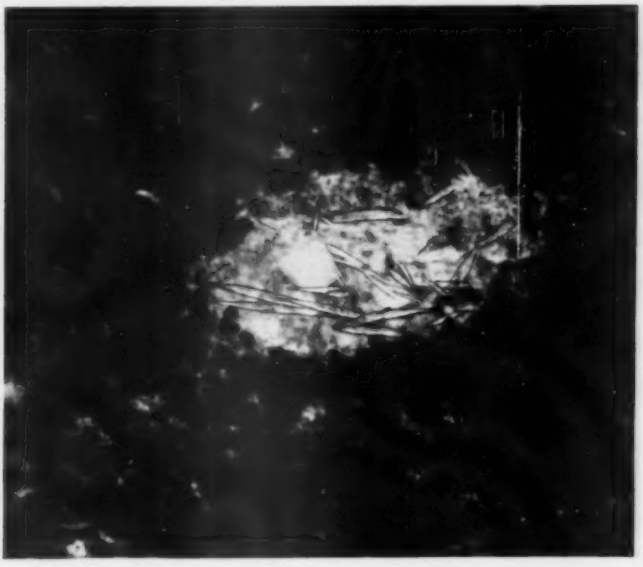

FIG. 4. CASE 3 (SURG. PATH. NO. 21703). HIGH-POWER PHOTOGRAPH OF NEEDLE CRYSTALS (RETOUCHED) WITHIN A MASS OF DISINTEGRATED ARGENTAFFINE TUMOR CELLS

Microscopical note. The tumor is about 5 to 8 mm. in diameter and, when seen microscopically, is found to be situated in the muscularis and to extend through the peritoneal covering into the mesenteric fat in the region of its attachment to the appendiceal wall. The character of the

section does not permit one to say definitely that the submucosa and mucosa are free from tumor.

The character and arrangement of the tumor cells and their supporting fibrous framework do not vary from those of the tumors already described. The outer coat of muscle frays out as it passes into the tumor and finally becomes so disarranged that an organized muscular layer is no longer recognized.

When frozen sections are made and the tumor stained with Scharlach R and haematoxylin, the tumor cells are found loaded with large droplets of lipoids (Fig. 8). The fat content of the tumor is so pronounced and so characteristic that one can easily pick out in the section minute, isolated cell clumps, merely by the Scharlach stain. All of the cells are fat-laden, though the quantity of lipoids in different cell nests seems to vary appreciably, some con-

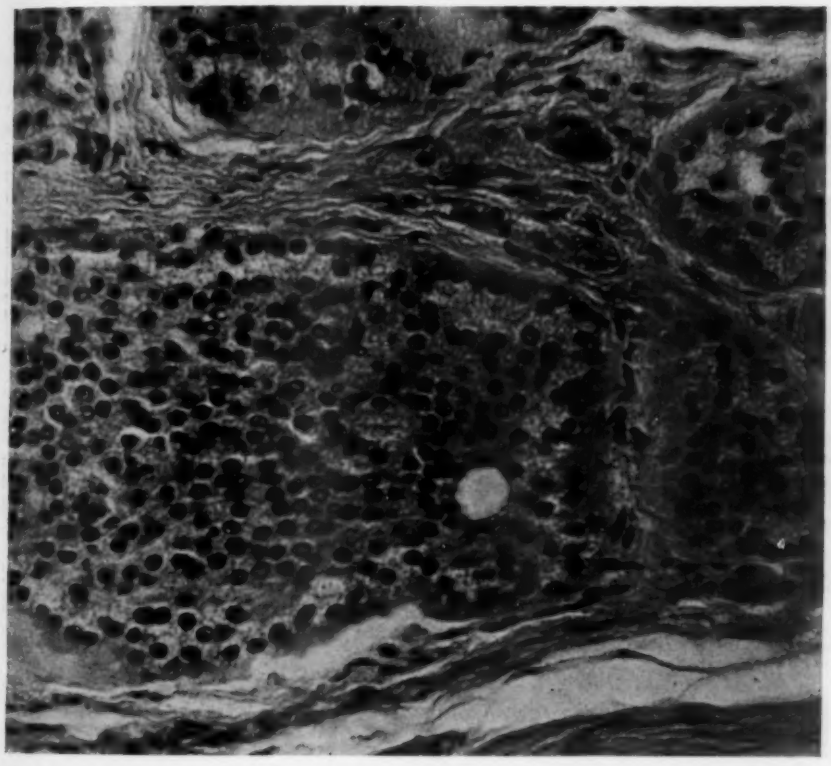

FIG. 5. CASE 4 (SURG. PATH. NO. 34485). HIGH-POWER PHOTOGRAPH OF ARGENTAFFINE TUMOR OF APPENDIX

A pseudo-glandular arrangement of the cells about a clear space, simulating a lumen, is seen in the large nest of cells. Ordinarily, as seen in Fig. 10 (Case 6), such spaces contain a small vessel surrounded by a narrow zone of connective tissue, or else hyaline cellular debris. It is obvious that the arrangement is not glandular. The peripheral cells of the nest have a finely granular cytoplasm. The central cells are vacuolated. The stroma is hyaline connective tissue.

taining small quantities and others a great abundance of the Scharlach-staining globules.

When stained with the Wilson blood-stain the granules of the tumor cells appear coarse and reddish-brown in color.

When small blocks of the tumor are impregnated with silver nitrate and then treated with ammoniacal silver solution, according to the method of Hasegawa[1] (44), the tumor cells show in a characteristic fashion the argentaffine granules (Fig. 7). These granules are seen better preserved in the cells at the periphery of the cell nests, where they are so abundant as to produce a black zone. The cells in the cen-

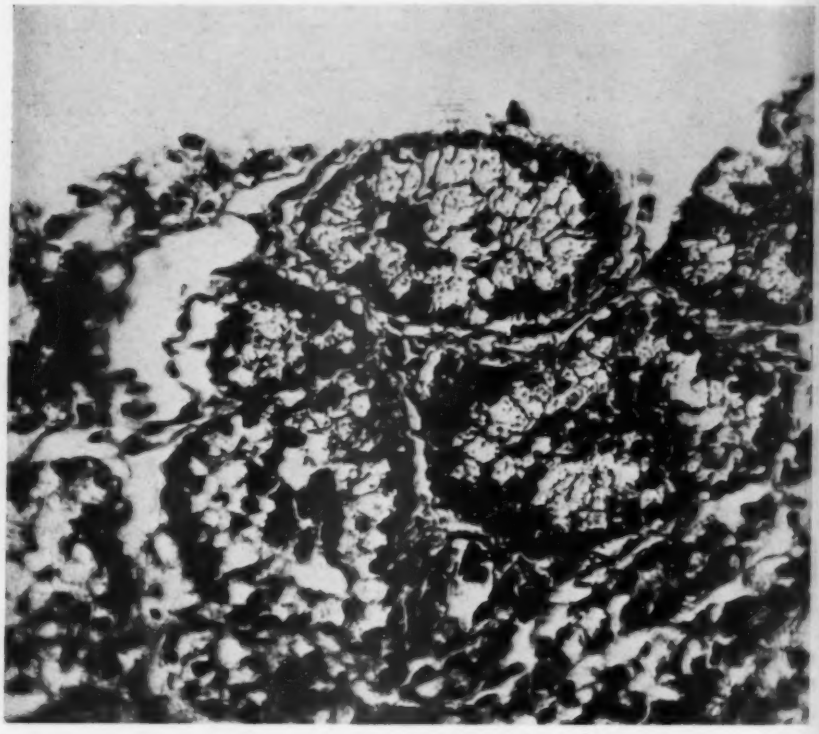

FIG. 6. CASE 4 (SURG. PATH. NO. 34485)

Same tumor as pictured in Fig. 4 and Fig. 5, stained by silver impregnation method. The peripheral cells of the tumor nests and a few of the central cells contain great quantities of argentaffine granules. The majority of the central cells are vacuolated and contain chiefly lipoids, as shown in Fig. 8.

With the combined Van Gieson and Verhoeff stains, and the Mallory connective-tissue stain, no connective-tissue stroma can be demonstrated in any of the cell nests. The small capillary vessels which are occasionally seen surrounded by masses of tumor cells are brought out conspicuously with these stains.

[1] Small blocks of tissue are fixed in formalin and subsequently washed in running water. The blocks are then allowed to

ter of each nest show some granulation, but they contain largely lipoids which are lost in the technical procedure, leaving vacuolated spaces where the lipoids were originally. The cell membrane still persists, however, and is clearly seen giving the whole cell nest a honeycomb-like appearance.

Case 5. Surgical Pathology No. 15978. Figure 8.

Clinical history. The patient was a

FIG. 7. CASE 4 (SURG. PATH. NO. 34485)

Same tumor as pictured in Fig. 5 and Fig. 6, stained with haematoxylin and Scharlach R (frozen section). The tumor nests are graphically indicated by the great quantity of lipoids which appear as black clumps. The cell forms are completely hidden by the stained lipoids.

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remain in a 2 per cent soution of silver nitrate for 24 hours, at 37°C., being always kept in the dark. The silver solution is washed out by rinsing in distilled water for a few minutes. The washing is followed by an ammoniacal silver solution for 24 hours at 37°C., in the dark:

2 per cent $AgNO_3$..... 10 cc.
$Ca(OH)_2$ or NaOH..... 7 drops
NH_4OH drop by drop until precipitate dissolves.

Wash in running water and dehydrate quickly. Embed in paraffin and cut sections as thin as possible. Mount in balsam.

white male, aged 12 years, who three weeks previous to his admission to the hospital had suffered from an abscess located in the subcutaneous tissue and the muscle of the right lumbar region. One week before admission for the present illness he had headaches and slight fever, associated with sharp pains, beginning in the right hypochondrium and radiating to the right lower quadrant of the abdomen. Nausea and vomiting were associated with the abdominal pain. When the patient was examined, classical signs of an acute appendicitis were found. At operation a perforated appendix was found which had given rise to an abscess in a retrocaecal position. The appendix was removed and the abscess drained. Convalescence was uneventful.

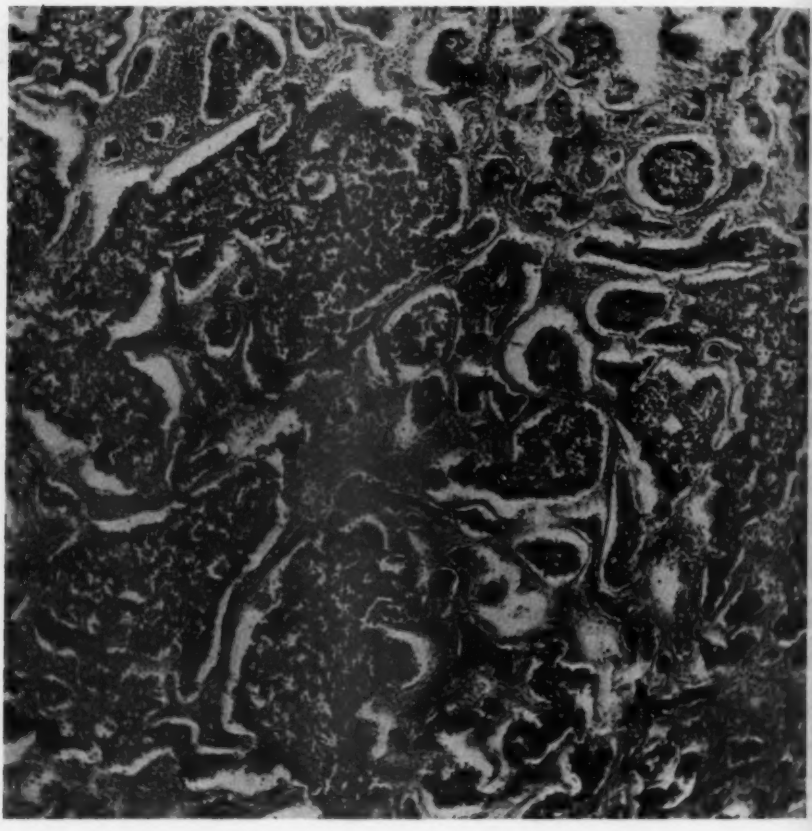

FIG. 8. CASE 5 (SURG. PATH. NO. 15978). ARGENTAFFINE TUMOR OF THE APPENDIX

The arrangement of the tumor cells is somewhat different from that in the other five cases. A festoon-like arrangement of cell cords forms the tumor masses. In between these cords is a fine connective-tissue framework. A coarser connective-tissue stroma forms the rest of the tumor.

Gross description. The appendix is 7 cm. in length and about 2 cm. in diameter,

the tip being larger than the proximal end. Three centimeters from the tip the lumen is completely obliterated by a yellowish tumor, 1 cm. long and 5 mm. in thickness.

Microscopical note. The section does not show the complete wall of the appendix, spect to the various parts of the wall of the organ. It seems, however, that the tumor lies in the mucosa, the epithelium of which has been destroyed, and has extended through the submucosa, muscularis and well into the mesenteric fat.

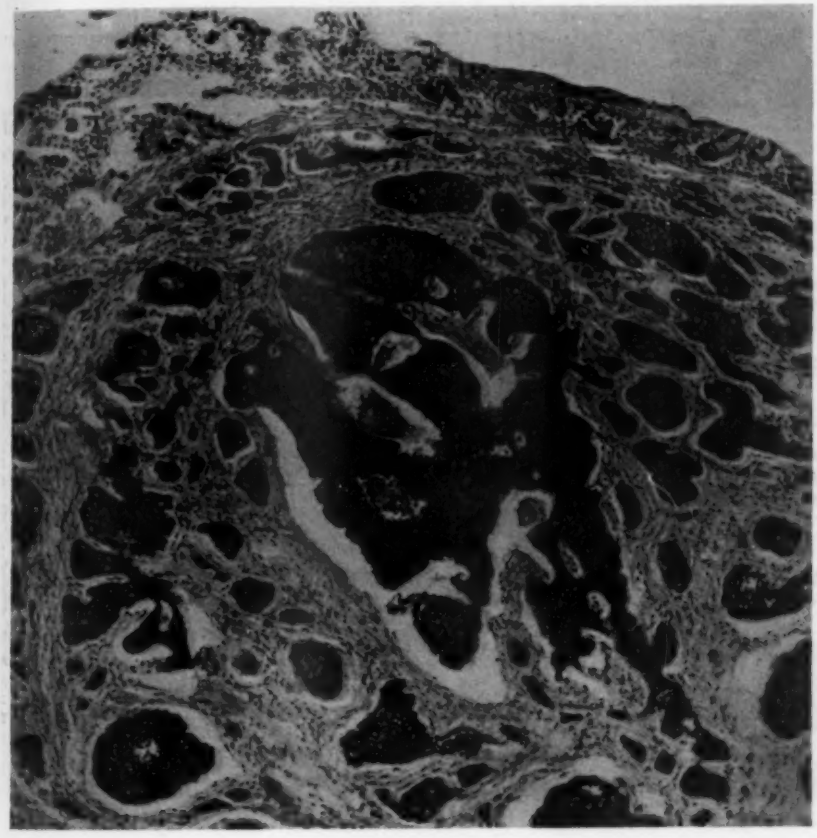

FIG. 9. CASE 6 (AUTOPSY NO. B.V.-1669). ARGENTAFFINE TUMOR OF THE ILEUM

The tumor lies largely in the submucosa, though a few nests can be found just outside the muscularis mucosæ. Spaces are seen within the cell masses which suggest the lumen of a gland-like arrangement of the cells. Close examination shows that these spaces contain a thin-walled vessel or hyaline cellular debris.

because of an associated acute appendicitis with abscess formation. It is therefore difficult to determine the actual borders and hence the location of the growth with re-

The general morphology of the cells which form the tumor together with their staining reaction is identical with that of the tumors described in detail above. The

arrangement of the cells is also essentially the same, though there is a variation seen here which is of interest. The usual solid nests and strands of cells infiltrating fibrous tissue and the muscle coats are numerous. The peculiarity of structure which has not been seen in the other cases consists of chains of cells of oval form, the chains being so arranged that their long axes are parallel. These chains seem to fold upon themselves, forming a festoon-like structure, the cell columns being separated by spaces (Fig. 9). In the spaces can be seen bits of pink-staining material which suggest necrotic cellular debris.

In the areas in which the tumor cells are packed together in solid masses, a peculiar delicate network of very small spindle cells is seen. These cells do not have any of the chaacteristics of the cells of the tumor but are more like connective-tissue cells. They are so distributed as to suggest a very fine stroma within the cell masses, a feature which has not been observed in our other cases.

FIG. 10. NORMAL APPENDIX. SILVER IMPREGNATION METHOD OF HASEGAWA

The argentaffine cells are made conspicuous in the depths of the crypts of Lieberkühn by their black granules.

Case 6. Autopsy No. B. V. 1669. Figure 9.

Clinical history. The patient was a man, aged 68 years, who for seven years had been

known to have pulmonary tuberculosis. He had had several admissions to the hospital because of this condition. The final illness was a flare-up of the lung trouble which was followed by generalized tuberculosis. The clinical diagnosis was advanced pulmonary tuberculosis, emphysema, arteriosclerosis and hypertension.

Anatomical diagnosis. Pulmonary tuberculosis, generalized miliary tuberculosis, chronic mitral endocarditis, myocarditis, infarcts of left kidney, prostatic hypertrophy, chronic prostatitis with thrombosis of prostatic vein, acute cystitis and bilateral pyelitis, "benign epithelioma" in the wall of the ileum.

Microscopical note. The tumor is a small nodule, 2 mm. in diameter, located in the submucosa of the ileum. The mucosa is well preserved over the tumor nodule, except that it shows some atrophy of the villi, and a loss of epithelial covering. The latter is probably due to the technical procedures. The muscularis mucosae is thinned out but with some difficulty can be traced along over the tumor. No direct connection is found between tumor and the glands of the mucosa in this single section, although the tumor extends well up to the zone of the deepest crypts, and is separated from the mucosa only by a very few strands of connective tissue and smooth muscle. Toward the outer edge the tumor has begun to invade the inner coat of the muscle, which, however, shows chiefly a thinning out and an atrophy due to pressure from the tumor. The other muscle coat appears normal, as does the peritoneum. The cellular structure and arrangement of the cells are the same as already described in the preceding cases and are quite characteristic of the argentaffine tumor.

DISCUSSION

It is evident from the description of the above cases that one is dealing with a tumor of peculiar morphological, as well as chemical, character, and that this tumor is beyond question different from the usual adenocarcinoma derived from the cylindrical cells of the epithelium of the intestinal tract. Differentiation between the tumors under question and the adenocarcinomas from a clinical standpoint has been made clear by former studies, and this difference is well recognized. It is possible and, in fact, quite easy, to make this differentiation on a purely morphological basis, without calling to aid the more complicated and time-consuming process of silver impregnation. In all of our cases the arrangement of the cells, the character of the cytoplasm, and the general relationship of the tumor to the intestinal wall, form a very constant and reliable basis for differential diagnosis.

It would be very thoughtless, indeed, not to realize fully that these tumors represent simply one group of neoplasms which occur in the gastro-intestinal tract, and have their origin in the epithelial cells of the mucosa. True cylindrical cell adenocarcinoma, as everyone knows, may occur in the small intestine and the appendix, although, in comparison with the frequency in other parts of the gastro-intestinal tract, they are rare.

All of the six cases, which have been described above, are characteristic argentaffine tumors, as shown by their morphology, staining reactions, location, and general biological behaviour. Three of the tumors were found at autopsy and the other three in appendices which were removed because of symptoms pointing to an inflammatory condition in the appendix, but without any evidence whatever of the presence of a tumor; and so it is that the cases here conform to the usual accidental finding of the tumor, which is so generally spoken of throughout the literature. In no case were secondary tumors found anywhere. Extension

of the tumor cells into all the coats of the intestinal wall was common to all, and further extension into the mesentery was seen in two of the cases.

The presence of a crystalline substance in some of the cell nests as seen in our Case 3 is of interest because of the lipoidal content of the cells as shown by the Scharlach R stain. It is suggested that the crystalline substance had its origin in the lipoids of cells which have undergone destructive changes. Were there definitely formed lumina in which these crystals occur, one might think of them as a secretion product, a feature which would give support to Masson's idea concerning the endocrine character of the tumor. However, in the absence of a glandular structure, such a viewpoint can hardly be maintained, and the significance of the crystalline substance, as well as of the lipoids and granules, remains a problem.

In all six tumors the stroma is quite the same, being composed of hyaline connective tissue, a fair number of blood vessels (not extraordinary), rather inconspicuous lymph spaces, nerve bundles of widely varying numbers, and, toward the periphery, some smooth muscle. This muscle is almost always seen in direct continuity with the muscularis mucosae or the inner muscular coat of the organ. It seems most unlikely that it forms any part of the new growth.

Under the most careful study, morphologically and by staining methods, the tumors in this series reveal nothing more than a slight superficial resemblance to the tissue of the islands of Langerhans. The characteristic arrangement of tumor cells and blood vessels described by Saltykow is not seen, nor can we find any definite and constant relationship between tumor nests and vessels. In none of the cases can tumor cells be found within blood vessels or in lymphatics. No evidence whatever is found for the support of the endothelial origin of the tumors, as suggested by Glazebrook and others.

It seems desirable, in conclusion, to call attention to a peculiar type of carcinoma of the small intestine which syperficially resembles the argentaffine tumors. This tumor is composed of nests and strands of cells which are not always columnar in form and which do not always from a glandular structure, although a characteristic alveolar arrangement can be made out in parts of of the tumor which alone differentiates it from the argentaffine tumors on a morphological basis. The cells may be seen in compact masses and it is here that they so closely resemble the argentaffine cells. The characteristic silver reaction, however, is absent, as are also the more minute characters of the cell structure. This tumor is a true adenocarcinoma and as such produces widespread metastases. One of Hasegawa's cases seems to belong to this group. There are other cases described in the literature as "carcinoids" with metastases, which may likewise be of similar character. The differentiation between the argentaffine tumors and this form of carcinoma is, of course, of the greatest importance. The carcinoma can usually be recognized without the use of the silver method, if careful microscopical studies are made.

The clinical aspects of the argentaffine tumors have been quite adequately described, so that no detailed

discussion of this phase of the subject seems warranted. Briefly, however, the clinical status, as revealed by the many case reports, is in summary as follows:

1. The argentaffine tumors occur throughout the length of the gastro-intestinal tract below the oesophagus.

2. They may be single or multiple.

3. They never produce symptoms or signs *per se*.

4. They are usually confined to the wall of the intestine but may extend into the mesentery and in rare cases to the lymph nodes. Metastasis to remote parts is extremely doubtful, if it ever occurs.

5. The frequency of occurrence in all appendices removed at operation is about 0.4 per cent.

6. They are most frequent in the appendix between the ages of 20 and 30, though they may occur at any age from ten days up.

CONCLUSIONS

1. Attention is called to the fact that the term *carcinoma* has been erroneously applied to a group of small tumors occurring either singly or in numbers in the appendix and small intestine, and which bear a morphological similarity to true carcinoma of these organs.

2. Six cases of so-called "carcinoma" of the appendix and small intestine are described, and the argentaffine or chromaffine character of the cells of these tumors, originally described by Gosset and Masson, confirmed.

3. The "carcinomas" or "carcinoids" of the appendix and small intestine have their origin in the cells of Kultschitzky and Schmidt of the crypts of Lieberkühn and, therefore, are tumors of the chromaffine system. They may be spoken of as argentaffine tumors, in view of the peculiar ability of the granules of the tumor cells to reduce an ammoniacal solution of silver.

4. The importance of differentiation between the argentaffine tumors and a peculiar form of adenocarcinoma of the appendix and small intestine, which is superficially similar to the argentaffine tumors, is emphasized.

5. Attention is called to the general harmless character of the argentaffine tumors.

6. A review of the literature is given tracing the development of the various opinions regarding the so-called "carcinomas" or "carcinoids" of the gastro-intestinal tract.

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Book Reviews

The Medical Sciences in the German Universities. Translated from the German of Theodor Billroth. With an introduction by William H. Welch. $3.50. (*New York, The Macmillan Co., 1924.*)

This is a book on medical education written by the great German surgeon nearly half a century ago and now presented for the first time in an English translation. Not the least readable part of the book is its introduction which is written by Dr. Welch. The author presents his views of the educational situation and of the problems of his time, and its reading to modern ears has a strangely contemporary and familiar sound. A most interesting account is given of the historical development of medical education in the German speaking countries; of the gradual introduction of the various branches of study; and of how the latter came to be separated from one another with the foundation of the separate chairs. He gives a list of the distinguished occupants of these chairs and traces the historical sequence from master to pupil, analyzing the qualities of the men which made them great teachers, who attracted many pupils, and who were at the same time contributors to medical science. The picture which is drawn of the ideals and of the extraordinary development of the methods and opportunities for study and research in the German and Austrian medical centers of the time is illuminating, and removes any false pride in present day advances.

Some of Billroth's views would probably receive very scant consideration from some of the more advanced modern thinkers, for they are not always orthodox, and one fears could hardly be taken seriously by Professor Gottlieb. For example, he objects to mathematics in the preparation of medical students and states that only two or three out of a class of forty in the average *Gymnasium* could solve a mathematical problem unaided—and they were "usually fellows untalented in other ways, who have since become obscure schoolmasters." Of course no medical research can be taken seriously nowadays which does not contain at least one formula.

It is interesting to note that some modern proposals for reform in medical education are not particularly new. For example, the frequently proposed correlation of clinical and preclinical teaching early in the medical course was suggested as part of a plan for the revision of the medical curriculum in the University of Vienna as early as 1876 by a rather impractical Austrian emperor.

After all, the translator of this remarkable and intensely stimulating book is probably right in adding on to the original name the sub-title—"A Study in the History of Civilization." Like other educational developments, medical science can only proceed in step with the general cultural progress of the times—it will not often far outstrip it, despite the efforts and influence of those who consider themselves unusually discerning.

G. H.

The Theory and Practice of the Steinach Operation. By PETER SCHMIDT. 150 pp. 7/6. (*William Heinemann, London, 1924.*)

One recommends with great hesitancy the translation of Dr. Schmidt's book on the Steinach operation. The work is obviously that of a very energetic, practical and optimistic surgeon. The basis for the work is the report of eighty-four of his operative cases. One wishes that operative technique has been discussed in greater detail. The operator insists, however, on the importance of the technique and its great simplicity. Dr. Schmidt states that by vasoligation he has either cured or greatly helped the following con-

ditions in one or more patients: the cachexia of carcinoma, baldness, bad eyesight, hypertension, glycosuria, angina pectoris, chronic nephritis, the pain of aneurysm of the aorta, creaking of the cervical vertebrae, asthma, homosexuality, nymphomania, impotence, etc. One cannot but feel that he has been unable to separate, critically, the psychic from the organic effect in many cases. He states definitely that he considers the patient's symptoms more important that the readings of the mercury manometer in following the progress of hypertensive patients. The case records are often very incomplete. The translation is clumsy; such terms as "endocrine index" are alluring but meaningless. The cases are presented unsystematically. The results are not statistically analyzed, because the author feels that in this field of work such an analysis would be misleading.

The book introduces one to a fascinating branch of surgery. There may be great potentialities in this field, but there must be more and better controlled reports of cases than those of this ardent disciple before such brilliant results are accepted by the scientific world.

M. S. G.

The Medical Department of the United States Army in the World War. Vol. xi, Pt. 2. 827 pp. (*Washington Government Printing Office, 1924.*)

This volume of the history of the Medical Division of the United States Army affords a pleasant surprise to the reviewer. Instead of containing merely an uninteresting compilation of statistics, it has instructive chapters on empyema, maxillofacial surgery, and ophthalmology. The section on empyema is most comprehensive, and deals with the epidemiology, pathology, and treatment of the types seen after the epidemic of measles and influenza. The first chapter shows clearly that the incidence of empyema conforms with that of infections of the upper respiratory tract. The chapter by Lieut. Col. Keller, on the treatment of the refractory empyema cavities, gives a brief account and summary of his experiences at the Walter Reed Hospital. Each point made by the writer is illustrated with apposite case records. The section on maxillofacial surgery is particularly instructive. There are over 500 well selected and vivid illustrations. The text is full of practical suggestions for plastic surgery of the face. The entire volume is one that will be referred to frequently, more especially because it treats of conditions which are even more common to civil practice than in military surgery.

W. M. F.

The Primitive Archaic Forms of Inner Experiences and Thought in Schizophrenics. By Dr. Alfred Storch. 111 pp. $2.00. (*Nervous and Mental Disease Publishing Co., New York and Washington, 1924.*)

This book sets side by side instances of primitive customs and beliefs and examples of the behaviour and utterances of schizophrenics. No one can help but be impressed by the similarities between the two groups of facts. The author is careful to quote case-material extensively, and is content to leave the inferences to be weighed by the reader. The work is a valuable antidote to the one-sided psychoanalytic dogma, of which we are becoming so tired. Storch avoids the temptation to make play with the term "regression," as if the schizophrenic process followed the biogenetic law—backwards. The resemblance between the experiences of the mystic and the cosmic identification of the schizophrenic is impressive, but the author does not make the mistake which Thouless seems to commit (*Psychology of the Contemplative Life*) in stating that the mystic's self-absorption is dementia praecox. "Quality-complexes," of which much is made in this thesis, are no new discovery of the comparative method: they have long been familiar to academic psychologists in other terms—the "big buzzing confusion" and "implicit awareness" of child psychology. The translation has been well done, but "displacement" (p. 13) would surely be a better rendering than "distortion."

R. D. C.

The Clinical Examination of Surgical Cases. By J. Renfrew White, M.D. 134 pp. (*The Macmillan Co., New York.*)

This is a small volume written to aid students to train themselves in habits of careful and systematic examination of cases. The text is in outline form and simply enumerates all the examinations which one should make for various surgical conditions. There is practically no mention made of points in differential diagnosis. As in every syllabus, it is difficult for the author to indicate the relative importance of one method of examination over another. The book will be of value primarily to students who are just commencing their surgical training. M. F.

Diseases of the Heart. By H Vaquez. Translated into English by Dr. George Laidlaw. 743 pp. $8.50. (*W. B. Saunders Company, Philadelphia.*)

It is fortunate that we now have a translation of this book. The revision for the American edition brings it quite up to date, and it will be a valuable book of reference for all studying heart disease. This is true not only for novices but also for experienced students.

The value of text-books, especially new ones, is frequently limited by their being merely reiterations and rearrangements of the usual stereotyped descriptions and definitions. Those that are different—and this is one—are the simple accounts of the opinions of a close observer based on his long experience and his knowledge of the literature. This has the result of making interesting reading and stimulates first the beginner, since it gives him basic criteria such as he should get from any good clinical teacher of any subject, and secondly, those more experienced because they either agree with the conclusions of M. Vaquez, or disagree, which may do them more good. In all the sections other authorities are quoted systematically and clearly, the soundness of the various opinions is usually dealt with carefully and without prejudice, M. Vaquez giving his reasons for agreement or disagreement. Some of his statements seem old-fashioned and one's own would not always tally. An instance of this sort is the remark that aortic stenosis, of arterial origin, "may be due to syphilis, alcohol or lead poisoning, or to the abnormal rise of pressure provoked by these disorders" (page 386). Nor can we agree that angina abdominalis "results from a special localization of the organic lesions in the lower part of the thoracic aorta" (page 412). Nor does it seem consistent when he says, on page 435, that the anginous pain in coronary thrombosis is in the epigastrium. These are merely examples from one part—the kind of thing that stimulates discussion. The clearness and compactness with which his views and conclusions from life-long observation are stated give one that impression of solidarity which comes only from an obviously thoroughbred clinician.

All through, accounts of clinical cases are strikingly pictured, illustrating the point made. The practical side of cardiac diseases is done well and shows the touch of one who has had intimate knowledge of the various procedures.

Unlike a good many of the American editions of foreign books the translation is done into good English. T. O.

Recovery Record. By G. B. Webb and C. T. Ryder. 185 pp. $2.00. (*Paul B. Hoeber, Inc., New York, 1923.*)

Although the burden of the cure is upon the patient, he needs advice occasionally from the physician, and this little book aims to be a guide-book. An exposition, in lay language and definition, of some of the conspicuous symptoms and incidental complaints, the book includes a dissertation upon the hygiene of recovery and advises the patient as to the significance of the temperature and pulse reactions, the technique of taking and recording them, of diet and fresh air, of protecting others, of regular habits, of amusements, of simple care of constipation, anorexia, nervousness, sleeplessness, headache, hemorrhage and other true symptoms. In addition to the many helpful suggestions which should enable the patient to understand his sickness rationally, the authors have added some one hundred pages of temperature and pulse charts, so that the book may become for the patient an individual record book. Quotations from the classics and from modern works on tuberculosis embellish the chart pages. H. W. W.